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PROCEEDINGS
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VITAMIN B_6, A GROWTH SUBSTANCE FOR EXCISED TOMATO ROOTS[1]

By William J. Robbins and Mary Bartley Schmidt

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Communicated November 30, 1938

In earlier papers[2] we reported that thiamin (vitamin B_1) or the vitamin thiazole (4-methyl-5-β-hydroxy-ethyl-thiazole), in addition to sugar, mineral salts and water, is necessary for unlimited growth of excised tomato roots. We have maintained excised tomato roots in a solution of mineral salts, pure cane sugar and thiamin through 25 successive passages extending over a period of more than 2 years. In this solution growth is slow; the average dry weight of a single root rarely exceeds 10 mg. and is usually less.

When a light brown sugar is substituted for the pure cane sugar the growth is much improved, the average dry weight of individual roots reaching 70 or 80 mg. The beneficial effect of the impure sugar as compared to the pure sugar is in part because of its minerals but primarily because of the presence of vitamin B_6. We have demonstrated that this portion of the B complex has a marked beneficial effect upon the growth of excised tomato roots.

The roots with which we have worked came originally from seeds of a pink fruited variety of *Lycopersicon esculentum* from Mexico. The excised roots have been kept in culture since September 29, 1935, and represent two clones which respond alike. The roots were grown individually in 50 ml. of solution in 125 ml. Erlenmeyer flasks at a temperature of 20–25°C. and in diffuse light.

Experiments on the growth of excised tomato roots in a solution of minerals, pure cane sugar and thiamin supplemented with the ash of the brown sugar failed to show more than minor benefits from the addition of the ash. The ash was used in various amounts both before and after digestion with hydrochloric acid.

The addition of nicotinic acid, nicotinicamide[3] or White's "complete and optimal mixture of amino acids"[4] to a solution of minerals, pure cane sugar and thiamin was of slight or no benefit to the growth of the roots.

Through the courtesy of Merck and Co., a sample of crystalline vitamin B_6 isolated from natural sources was secured. Added to a solution of mineral salts, cane sugar and thiamin in amounts of 1 γ or 5 γ per flask it produced a marked increased in growth. The dry weights of roots grown individually for two months in a modified Pfeffer's solution containing 2% pure cane sugar and supplemented with light brown sugar ash, thiamin and vitamin B_6 are given in the following table. The light brown sugar ash was digested with HCl and added in amounts equivalent to the ash in a 0.4% solution, a concentration which had been found more favorable than more dilute or more concentrated solutions. The amounts of thiamin and of vitamin B_6 given in the table are those added to each flask containing 50 ml. of solution. In each instance the weight of the root is the average of 5 roots each grown individually in a 125 ml. Erlenmeyer flask.

PFEFFER'S SOLUTION CONTAINING 2% PURE CANE SUGAR PLUS	AV. WT. PER ROOT MG.	PFEFFER'S SOLUTION CONTAINING 2% PURE CANE SUGAR AND LIGHT BROWN SUGAR ASH PLUS	AV. WT. PER ROOT MG.
No addition	0.4	.....	..
5 γ Thiamin	3.4	5 γ Thiamin	7.6
5 γ Thiamin and 1 γ Vitamin B_6	16.1	5 γ Thiamin and 1 γ Vitamin B_6	14.5
5 γ Thiamin and 5 γ Vitamin B_6	15.7	5 γ Thiamin and 5 γ Vitamin B_6	22.4
1 γ Vitamin B_6	1.8	.....	..
5 γ Vitamin B_6	1.3	.....	..

No growth occurred in Pfeffer's solution plus pure cane sugar, and very little in the same solution supplemented with 1 γ or 5 γ of vitamin B_6 per flask; the addition of 5 γ of thiamin per flask to the Pfeffer's solution plus pure cane sugar permitted some growth; the addition of both thiamin and vitamin B_6 to the mineral-carbohydrate solution produced a marked increase in growth which was somewhat improved in the solutions containing thiamin and 5 γ of vitamin B_6 by the addition of brown sugar ash which had been digested with hydrochloric acid.

We believe that in a mineral salt-sugar solution supplemented with thiamin our strain of excised tomato roots synthesizes vitamin B_6 in amounts sufficient to permit slow growth but not in quantities adequate for rapid growth. Thiamin (or the vitamin thiazole) is the limiting factor for growth in a mineral salt-sugar solution, but vitamin B_6 is the limiting factor in a solution of mineral salts, sugar and thiamin.

We assume that the sample of vitamin B_6 which was available was not contaminated with an unknown growth substance; its crystalline character would support that opinion. Until synthetic vitamin B_6 is available this assumption cannot be made, however, with entire certainty. We assume also that the response of the excised tomato roots in solution cultures is

essentially that of attached roots in the soil under normal conditions. The validity of this assumption also must wait experimental confirmation. If these two assumptions are correct, then it would appear that the tomato root depends upon the top not only for carbohydrate and thiamin (or the vitamin thiazole) but for vitamin B_6. The parasitic relation of the root to the shoot is more complex than has hitherto been believed. Whether more growth substances than thiamin and vitamin B_6 are involved in the growth of the tomato roots with which we have worked remains to be seen.

Vitamin B_6 is an important growth factor for rats; a deficiency causes a marked acrodynia type of dermatitis.[5] Our observations show that vitamin B_6 is important for the growth of plants also; it may prove to be as generally significant for living organisms as thiamin.

[1] Supported in part by a grant from the American Philosophical Society.

[2] William J. Robbins and Mary A. Bartley, *Sci.*, **85**, 246–247 (1937); *Proc. Nat. Acad. Sci.*, **23**, 385–388 (1937); William J. Robbins and Mary B. Schmidt, *Bot. Gaz.*, **99**, 671–728 (1938).

[3] B. C. J. G. Knight, *Biochem. Jour.*, **31**, 731–737, 966–973 (1937).

[4] P. R. White, *Plant Physiol.*, **12**, 793–802 (1937).

[5] G. A. Emerson, Ali Mohammad, Oliver H. Emerson and Herbert M. Evans, *Jour. Biol. Chem.*, **124** 377–383 (1938).

CHEMICAL RESTORATION IN NITELLA. III. EFFECTS OF INORGANIC SALTS

BY W. J. V. OSTERHOUT AND S. E. HILL

LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Communicated November 25, 1938

The removal of irritability[1] and its restoration by certain organic substances have been mentioned in previous papers.[2] Attempts at restoration by inorganic salts gave no results in earlier studies but in recent experiments on a different lot of cells[3] have met with more success. The time required is much longer than for the organic substances previously mentioned and a different mechanism may be involved. It seems possible that the salts may cause a movement of the substance (or group of substances), called for convenience R_A, from the sap to the protoplasm, thereby restoring the irritability.

Cells[4] which had been kept in distilled water until irritability disappeared (2 to 6 days) were placed in 0.01 *M* NaCl and at intervals were taken out and tested by electrical stimulation (500 mv. D.C. for 1 second). It was found that an exposure of 5 minutes or more to NaCl was necessary to restore irritability.

The action curve was often single peaked[5] but the usual form was frequently observed. In many cases recovery took place suddenly giving "square topped" action curves.[5]

In some cases only part of the cell was exposed to NaCl and it was then found that irritability was restored only in this region and that immediately adjoining it.[6]

In some cases cells kept in Solution A[7] showed no irritability.[8] In these, as in leached cells, exposure to 0.01 *M* NaCl restored irritability and prolonged exposure (1 to 2 hours) produced quick action currents[9] (lasting about a second) and after still further exposure the irritability temporarily disappeared but after an interval normal slow action currents (lasting 15 to 30 seconds) appeared.

The leaching in distilled water removed not only the irritability but also the potassium effect (i.e., the negative change in P.D. caused by substituting KCl for NaCl). During the exposure to NaCl the electrical stimulation was usually followed by an application of 0.01 *M* KCl to see if the potassium effect had been restored. Such restoration was frequently found[10] but only after an action current. It is therefore probable that the action current was responsible since it is known that it can restore the potassium effect.[11]

Let us now consider certain experiments with the inorganic salts found in blood. The statement in a previous paper[12] that these salts do not restore irritability was based on experiments with cells which have been designated as Lot A and which differed in some important respects from those studied later which will be called Lot B.[3] In Lot B the inorganic salts of blood[13] were able to restore irritability in many cases but the time required was much longer than that for the oxalated blood plasma. The latter could restore irritability in 5 seconds in cells that required several minutes for restoration with the inorganic blood salts.

As stated previously[12] the time required with blood plasma varied considerably and this was true of the inorganic salts.[14]

After restoration by the inorganic salts of blood the forms of the action current resembled those mentioned earlier in this paper as the result of restoration by NaCl; those produced by the blood plasma were mostly normal (double peaked).

The behavior of the potassium effect was much the same as after exposure to 0.01 to 0.03 *M* NaCl.

Discussion.—Irritability may be rapidly restored by certain organic substances,[15] e.g., in a few seconds by blood, urine and saliva, and substances extracted from the distilled water in which *Nitella* has been standing. But NaCl requires a much longer time and it seems possible that this might suffice to allow a variety of changes to take place in the cell. It might, for example, cause a movement of water from the sap to the proto-

plasm and this might be accompanied by a movement of R_A. The inner protoplasmic surface Y (adjoining the vacuole) is constantly in contact with R_A contained in the sap but this is very different from having R_A on both sides (this has been noted in a previous paper[16]).

Exposure to NaCl may contribute to the restoration of irritability by raising the conductivity of the protoplasm[9] so that less voltage is required to produce a stimulating current.[1] The conductivity of the cellulose wall is also raised but this is evidently of little importance since it is completed in a few seconds; but to restore irritability several minutes are required.

It is also possible that NaCl may act as a catalyst to promote the formation of R_A in the protoplasm.

What is said regarding NaCl applies to 0.01 M Na_2SO_4. It likewise applies to the inorganic salts of blood which seem to be more effective than NaCl at corresponding total concentrations even when the blood salts are made up without calcium. Calcium alone can restore irritability and the potassium effect slowly, probably by preventing R_A and R_P from dissolving out of the cell surface.[17] (R_P is an organic substance which appears to be responsible for the potassium effect.)

Until the mechanism of irritability has been cleared up it does not seem useful to make further hypotheses.

Summary.—Nitella cells which have lost their irritability may have it restored by exposure to 0.01 M NaCl or to Na_2SO_4 but the time required is so much longer than for certain organic substances that a different mechanism may be involved. Possibly NaCl causes a movement of organic substances from the sap into the protoplasm.

The inorganic salts of blood are somewhat more effective than NaCl.

[1] By irritability is meant the production of action currents by electrical stimulation when the recording contact is 1 cm. or more from the cathode. A contact at the cathode usually shows some sort of response by the cell if the stimulating voltage is high enough, but this is not propagated along the cell. Exposure to 0.01 M NaCl lowers the voltage threshold for this response but the current is not diminished to the same extent because NaCl increases the conductivity of the cell.

[2] Osterhout, W. J. V., and Hill, S. E., *Jour. Gen. Physiol.*, **17**, 105 (1933–1934), *Proc. Soc. Exptl. Biol. Med.*, **32**, 715 (1934–1935); Osterhout, W. J. V., *Jour. Gen. Physiol.*, **19**, 423 (1935–1936).

[3] These cells may be called Lot B to distinguish them from the earlier cells which will be called Lot A. Cf. Hill, S. E., and Osterhout, W. J. V., *Jour. Gen. Physiol.*, **21**, 541 (1937–1938).

[4] The experiments were performed on *Nitella flexilis*, Ag., using the technique described in former papers (cf. Hill, S. E., and Osterhout, W. J. V., *Jour. Gen. Physiol.*, **21**, 541 (1937–1938)). Temperature 20° to 25°C.

[5] For single peaked curves see Hill, S. E., and Osterhout, W. J. V., *Jour. Gen. Physiol.*, **22**, 91, Fig. 7 (1938–1939). For square topped curves see Osterhout, W. J. V., and Hill, S. E., "Some Ways to Control Bioelectrical Behavior," in *Cold Spring Harbor Symposia on Quantitative Biology*, Cold Spring Harbor, Long Island Biological Association, **4**, 43, Fig. 3 (1936).

[6] In this case cup technique was employed (cf. Hill, S. E., and Osterhout, W. J. V., *Jour. Gen. Physiol.*, **19**, 307 (1935–1936)) and some of the cups were filled with 0.01 *M* NaCl and the rest with distilled water.

[7] Osterhout, W. J. V., and Hill, S. E., *Ibid.*, **17**, 87 (1933–1934).

[8] This condition is often found in freshly collected cells in late spring and early summer (cf. Osterhout, W. J. V., and Hill, S. E., *Ibid.*, **17**, 105 (1933–1934)).

[9] Hill, S. E., and Osterhout, W. J. V., *Ibid.*, **22**, 91 (1938–1939).

[10] There was frequently a delay, cf. Hill, S. E., and Osterhout, W. J. V., *Ibid.*, **22**, 107 (1938–1939).

[11] Osterhout, W. J. V., and Hill, S. E., *Ibid.*, **18**, 681 (1934–1935).

[12] Osterhout, W. J. V., *Ibid.*, **19**, 423 (1935–1936).

[13] These were made up as follows:

Na_2CO_3	0.0147 *M*	$MgCl_2$	0.001 *M*	KCl	0.005 *M*
Na_2HPO_4	0.001 *M*	NaCl	0.128 *M*	$CaCl_2$	0.0025 *M*
		Total Cl = 0.140 *M*			

This mixture was diluted with 4 parts of water to correspond with the dilution of the blood plasma (cf. reference 12).

[14] In some cases this was much briefer than with 0.01 or 0.03 *M* NaCl.

[15] Osterhout, W. J. V., and Hill, S. E., *Proc. Soc. Exptl. Biol. Med.*, **32**, 715 (1934–1935); Osterhout, W. J. V., *Jour. Gen. Physiol.*, **19**, 423 (1935–1936).

[16] Hill, S. E., and Osterhout, W. J. V., *Ibid.*, **21**, 541 (1937–1938).

[17] Osterhout, W. J. V., and Hill, S. E., *Ibid.*, **17**, 105 (1933–1934).

PHOTOMICROGRAPHS OF THIN BONE SECTIONS BY THE USE OF FLUORESCENCE X-RADIATION

BY ELMER DERSHEM

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Communicated November 23, 1938

The x-ray photography of thin microtome sections requires the use of soft x-rays in order that the absorption in the specimen may be sufficient to provide photographic contrast. For such work low voltage x-ray tubes, sometimes called grenz ray tubes, have been used. These operate at potentials of 4000 to 10,000 volts and are provided with thin windows made of low atomic weight elements to permit the passage of weakly penetrating radiation. Such tubes are not commonly available and even so the radiation is much too penetrating.

However, much better results may be secured with an ordinary x-ray tube provided the rays from this tube are caused to pass through a thin sheet of material containing a properly chosen element. The atoms of this element will absorb part of the incident radiation, then emit fluorescence x-radiation which is practically confined to three or four closely adjacent wave-lengths characteristic of the element. This radiated body thus

becomes a source of nearly monochromatic x-rays, much more nearly monochromatic than radiation from an x-ray tube.

Each element has an x-ray absorption wave-length limit such that x-rays whose wave-lengths are just shorter than this value are much more absorbed

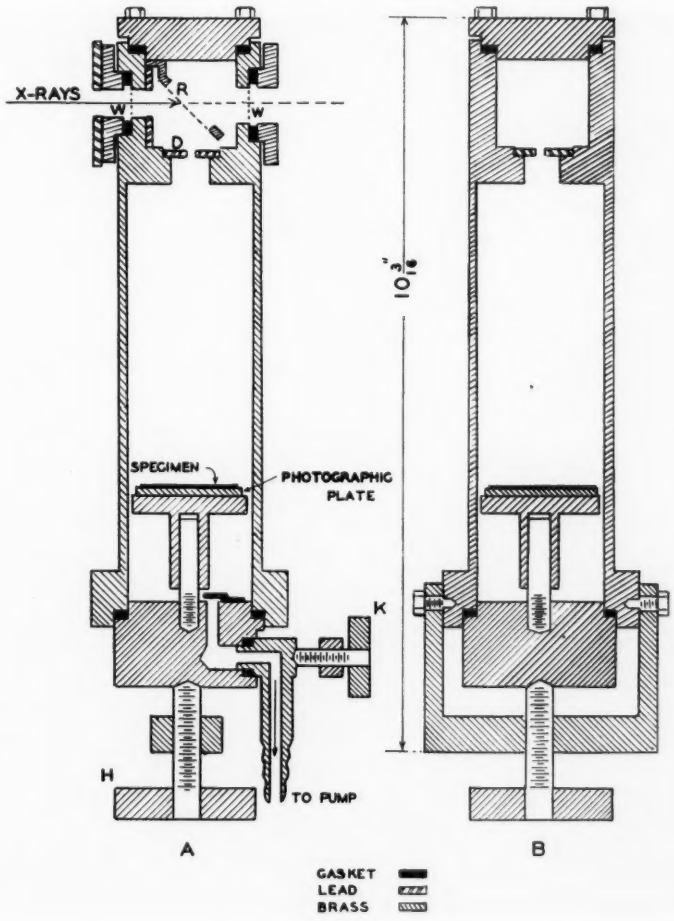

FIGURE 1

by the atoms of this element than rays of longer wave-length. Hence it is usually possible to choose an element for the fluorescent radiator such that its characteristic radiation is selectively absorbed by the atoms in the specimen.

The critical absorption limit of calcium is at 3.06 Å while the weighed average wave-length of scandium fluorescence radiation is 3.02 Å. Hence calcium very strongly absorbs this radiation. In fact the absorption of the 3.02 Å scandium radiation by a thin film of calcium may be one to

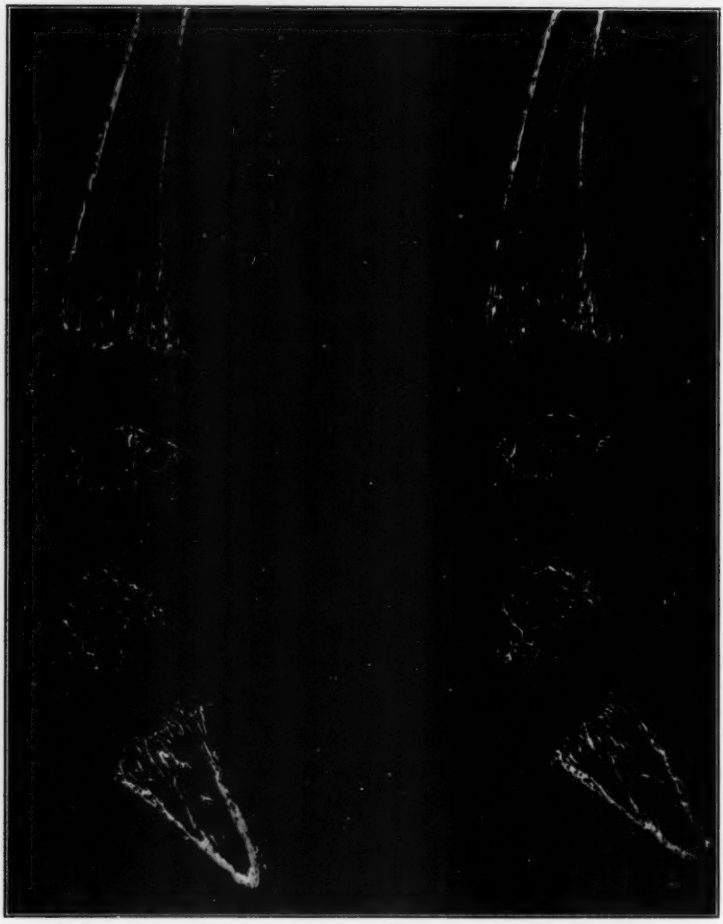

FIGURE 2

two hundred times as great as would be the case for radiation slightly longer than 3.06 Å in wave-length.

A camera permitting the use of such radiation was constructed and cross-sections of it at right angles to each other are shown in parts *A* and

B of figure 1. X-rays from any convenient x-ray tube pass through thin aluminum windows marked *W* and also through the radiator *R*. This radiator was made of thin onion skin paper to the under side of which a thin layer of the radiating material, in this case scandium oxide, was attached by mixing it with a thin celluloid cement.

The fluorescence radiation from the radiator *R* passed downward through the diaphragm *D* and thence through the specimen to the photographic plate. The specimen was held firmly against the plate by means of a clamp not shown in the sketch. X-rays of these wave-lengths are very strongly absorbed by passage through air, hence the camera was made airtight and

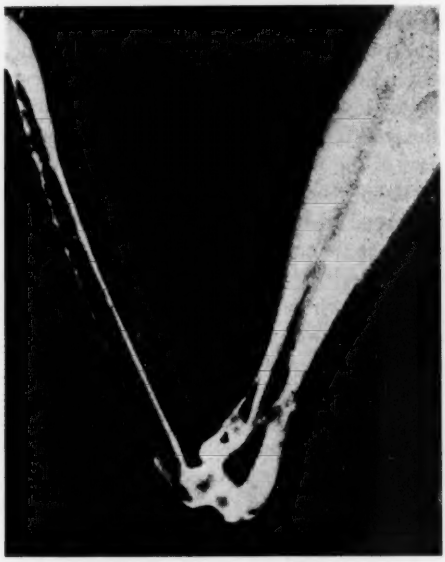

FIGURE 3

evacuated by a Cenco oil pump. The holder for the specimen and the photographic plate is attached to the lower end block of the camera and may be withdrawn by loosening the screw with the knurled head *H* and swinging aside the yoke to which it is attached. The camera may be loaded in the dark room and then sealed by compressing the gasket between the main tube and the end carrying the specimen holder, by means of the screw *H*. The camera may then be supported on a laboratory stand in such a position that x-rays from any available tube pass through the windows and the radiating material. The connection to the pump may be sealed to the camera by several turns of the knurled screw head *K* which

seats the pump connection against a rubber gasket. The radiator is attached to the upper end block of the camera. This end block is held against a rubber gasket by four screws. If these are removed the radiator may be removed and a radiator of some other element substituted.

Figure 2 shows a photomicrograph of a microtome section, 0.01 mm. thick, of the leg bones of a young rat. Due to the selective absorption of the radiation by the calcium every tiny particle of bone cast an unmistakable shadow on the plate. Eastman grenz ray plates were used. The exposure time was 40 minutes with a tube current of 10 milliamperes at 40,000 volts. The best results are to be secured with an x-ray tube voltage not greatly in excess of that required to excite the characteristic radiation of the element composing the radiator since the efficiency of the process by which general radiation from the tube is changed to characteristic radiation from the radiator is greatest in this case. Also, some radiation is scattered with either no change or only a slight increase of wave-length and if the x-ray tube voltage is high this will be of a short wave-length penetrating type which will mask the effect of the more strongly absorbed fluorescence radiation.

The same methods may be applied to problems in which other elements are involved. The proper element for use as a radiator with a given element in the specimen may be determined from a table of x-ray K emission and K absorption limit wave-lengths. The element to be chosen for the radiator should be the one whose K emission lines have wave-lengths just shorter than the K absorption wave-length of the element comprising the specimen. The fluorescence method may also be applied in cases where the specimen consists mainly of very light atomic weight elements such as carbon and oxygen whose selective absorption bands are far into the soft x-ray region. In this case the fluorescence method still yields a radiation much more absorbable than may be obtained directly from an x-ray tube unless the latter is of special design such that the specimen is placed within the x-ray tube itself.

Figure 3 shows a grasshopper leg photographed with scandium radiation. In this case the effects are mainly due to the general absorption of the soft radiation by light weight elements rather than selective absorption by calcium as in figure 2.

Other tests showed that for butterfly wings scandium radiation was too hard and better results could be secured with sulphur whose principal K emission wave-length was 5.36 Å.

The author is very deeply indebted to Dr. Franklin C. McLean and the Department of Physiology for supplying the microtome sections and financing the construction of the apparatus.

THE INDUCTION BY IRRADIATION WITH NEUTRONS OF HEREDITARY CHANGES IN MICE

By George D. Snell

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Communicated January 13, 1938

In a previous paper by Snell and Aebersold[1] the production in male mice by irradiation with neutrons of an initial period during which small litters are sired, followed by a period of complete sterility, followed, in turn, for the smaller doses, by a resumption of normal fertility, has been described. This paper is concerned with the appearance of sterility and of heredity changes in the descendants of the irradiated males.

The twelve irradiated males were from the C stock, homozygous for the mutant genes *b* and *c*. Before the onset of sterility they were mated as often as possible to females of the *P* stock (genotype $aabbCCddpps_cs_c$), or in a few cases to females of the *M* stock (genotype $aabbCCd_2d_2pps_cs_c$). F_1 animals were mated to mice of the *P* (or *M*) stock to give the second generation, designated as the F_2. F_2 mice of the appropriate sex were selected and back-crossed and to their F_1 parent to give the F_3 generation, among the individuals of which new recessive mutations may be expected to become phenotypically visible if any such have been induced by the treatment. The advantages of this type of cross in a search for induced mutations have been discussed in a previous paper by Snell.[2]

In order to make a rapid test of the semi-sterility of F_1 males, they were mated to females of the *Pch* stock as well as to the females from the *P* (and *M*) stocks. The *Pch* stock was selected because of its fertility.

The results of the tests for sterility and semi-sterility are summarized in table 1.

TABLE 1

	TOTAL NO.			NO. TESTED FOR FERTILITY		NO. STERILE		NO. SEMI-STERILE	
	♀	♂	?	♀	♂	♀	♂	♀	♂
F_1 test									
(*a*) Sired during pre-sterility period	16	25	3	10	23	0	3	1	3
(*b*) Sired during post-sterility period	13	10		2	5	0	0	0	0
F_1 control	43	28	5	19	3	0	0	0	0

The nature of semi-sterility in mice has been discussed in previous papers by Snell[2] and Snell, Bodemann and Hollander.[3] In the experiment here described, 62 F_2 and F_3 litters by F_1 control females averaged 9.3 young per litter, and 16 F_2 and F_3 litters by F_1 control males averaged 9.6

young per litter. The consistent production of litters considerably smaller than this may be regarded as evidence of semi-sterility due to a translocation. Experience has shown that a mouse whose litters average below 7 should be subjected to further tests for semi-sterility. Cases of sterility and semi-sterility found in the present experiment are listed below.

♂$R_1$8.—(Dose applied to father, 140 "r.") Fourteen litters of $R_1$8 by normal females averaged 6.0 young. Incompleted tests indicate that $R_1$8's tendency to produce small litters is transmitted to about one-half his progeny. In addition to the normal and semi-sterile offspring of $R_1$8, 5 out of 63 offspring that have been raised to maturity have shown conspicuously slow growth and have remained stunted throughout life. $R_1$8 is clearly semi-sterile. Further tests are in progress.

♂$R_1$12.—(Dose applied to father, 110 "r.") Fifteen litters of $R_1$12 by normal females averaged 3.3 young. Incompleted tests indicate that $R_1$12's tendency to produce small litters is transmitted to about one-half his progeny. Further tests are in progress.

♂$R_1$14.—(Dose applied to father, 110 "r.") Sterile. He was killed at eight months of age. There were no motile sperm in the sperm ducts. The testes were slightly below normal size. Sections show a very few spermatids, some in rather advanced stages, but no mature spermatozoa. The interstitial cells appear normal.

♂$R_1$28.—(Dose applied to father, 110 "r.") This male though placed with six different females produced only one litter, a litter of 2, which was not raised. He was killed at eight months. Many motile sperm were present in the sperm ducts.

♂$R_1$30.—(Dose applied to father, 110 "r.") Sterile. Male $R_1$30 was killed at eight months. A few immotile sperm were found in the left vas deferens, and a very few motile sperm in the left epididymis. No sperm were found in the right sperm tract. Sections of the testis showed a limited number of normal appearing spermatozoa to be present, but all of those observed were still attached.

♀$R_1$58.—(Dose applied to father, 140 "r.") Five litters averaged 4.4 young. The semi-sterility is definitely transmitted. Further tests are in progress.

♂$R_1$61.—(Dose applied to father, 120 "r.") Sterile. No sperm were present in the sperm ducts. Sections of the testes show spermatogonia and spermatocytes but no spermatids. Many of the tubules show hyaline degeneration of the germinal epithelium. The interstitial cells are normal or slightly increased in number.

In addition to the above, one test female, $R_1$2, produced nine litters averaging 5.7 young; one test male, $R_1$16, produced one litter of 4 and was then lost; one test female, $R_1$57, produced 4 litters averaging 6.8 young and was then discarded; one control female, $R_1$64, produced 3 litters

averaging 6.7 young and was then discarded; one control female, $R_1$65, produced 13 litters averaging 5.9 young; one test F_1 of unrecorded sex, $R_1$90, was found with a tail one-half normal length, shown by examination under the microscope to be a congenital defect and not the result of eating by the mother, but was missing three days later. Test female $R_1$2 and control female $R_1$65 were tested further by matings of their progeny. Of 10 tested sons and daughters of $R_1$2, only 1, a daughter, produced litters below normal size, and 3 grandsons by this daughter were normal. Fifty-one litters by 9 sons and daughters of $R_1$65 averaged normal size. Females $R_1$2 and $R_1$65 were, therefore, probably genetically normal despite the small size of their own litters. Similar cases among F_1 females have been noted previously by Snell[2] in the control group of a similar experiment. Aside from the above, all F_1 mice were phenotypically normal and produced litters averaging more than 7 young.

In the search for recessive visible mutations, 33 F_1 experimental animals (13 ♀ and 20 ♂) were tested. Back-cross matings between these mice and 83 of their offspring (17 sons and 66 daughters) yielded 842 F_2 mice. The majority of these were observed at birth and again at about three weeks of age. No mutations were found, though there were a few minor variations that failed to reappear. Twenty-five control mice mated to 39 of their offspring gave 467 F_2 mice, all normal.

Summary and Conclusions.—Out of the 44 F_1 animals in the experimental group there are thus 3 proven semi-sterile individuals (♂$R_1$8, ♂$R_1$12, ♀$R_1$58), 1 almost completely sterile male ($R_1$28) and 3 completely sterile males with abnormal spermatogenesis ($R_1$14, $R_1$30, $R_1$61). There are no cases of complete sterility and probably no cases of semi-sterility in the control, and while there are insufficient F_1 males in the untreated group to furnish an adequate control for the sterile males in the experimental group, the control groups of two other comparable experiments by Snell[2] and Hertwig[4] serve to show that in F_1 males from untreated parents, sterility is very rare. In quite extensive tests, no evidence was found for the occurrence of recessive visible mutations following irradiation with neutrons.

The similarity of the results found by Hertwig[4] and Snell[2] following irradiation with x-rays and the results here reported following irradiation with neutrons is noteworthy. Both treatments result in the production of translocations and of sterile F_1 males, and both fail to produce recessive mutations, at least in detectable numbers. Snell[2] found that a dose of about 700 r of x-rays applied to male mice caused translocations in about 33% of their offspring. This is a much higher incidence than the 9.1% (or 15.1% if ♂$R_1$28 and ♂$R_1$16 are included) found in the present experiment among animals sired during the initial fertile period. Yet Snell and Aebersold found that the reduction in F_1 litter size during the initial fertile

period was about the same in the two cases. Only further tests can show whether or not this represents a real difference between the effects of the two treatments.

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IMAGINAL DIFFERENTIATION INAUGURATED BY OXYGEN IN DROSOPHILA PUPAE

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The imaginal differentiation of the abdominal ectoderm of a Drosophila pupa depends upon a factor localized in the anterior part of the pupa. This factor acts during a critical period in pupal development. When the anterior part of the pupa is removed by means of a ligature before the critical period, the abdominal ectoderm is incapable of differentiation. On the other hand, imaginal differentiation takes place when the anterior part of the pupa is removed after the critical period.[1] Furthermore, it has been found that the differentiation of an eye anlage transplanted into an abdomen also depends upon the action of the pupal differentiation factor.[1] Such a differentiation center localized in the anterior part has not only been demonstrated for flies but also for Lepidoptera.[2,3] Thus the action of the imaginal differentiation center seems to be necessary for the developmental processes leading to imaginal completion.

We have already said that eye transplants can differentiate only after they are activated by the pupal differentiation center. The same has been found in skin transplants in the Micro-Lepidopteran Galleria.[4] Since the transplanted tissues lie free in the body cavity it was thought that the surrounding blood carried an "imaginal metamorphosis hormone" which is responsible for the differentiation of the transplants. The

differentiation center in the anterior part of the pupa thus represents a center of inner secretion which at the critical period releases its stimulating agencies into the blood. This point of view, based only upon the fact that the transplanted tissue in the abdomen of the animal seems to have only blood connections in its new environment, was held especially by Piepho[4] in his work on skin transplants in Galleria, whereas we also considered other possible interpretations of such experiments.

The work reported here was carried out on *Drosophila hydei* and is only a preliminary account; complete details will be published elsewhere. Previous work (in press) on the differentiation center in this species gave the following results. The critical period for the differentiation of the abdominal ectoderm of *Drosophila hydei* lies at about 28 hours after pupation. The ectoderm of the remaining posterior part always stays undifferentiated when the anterior part is removed by means of the ligature

TABLE 1

		CONTROL IN AIR			IN OXYGEN		
NO. OF EXPER. SERIES	AGE OF LIGATURING IN HOURS AFTER PUPATION	% OF CASES COMPLETELY DIFFERENTIATED	% OF CASES PARTLY DIFFERENTIATED	% OF CASES NOT DIFFERENTIATED	% OF CASES COMPLETELY DIFFERENTIATED	% OF CASES PARTLY DIFFERENTIATED	% OF CASES NOT DIFFERENTIATED
F 86	48	50	50	0	100	0	0
F 82	43	20	80	0	100	0	0
F 76	41–42	0	90	10.2	100	0	0
F 77	40–41	50	50	0	100	0	0
F 71	39½	33	67	0	100	0	0
F 72	24	0	0	100	37.5	25	37.5
F 83	24	0	0	100	54.5	9	36.5
F 87	24½	0	0	100	50	33	17
F 88	22	0	0	100	0	50	50
F 79	21	0	0	100	8	50	42

before this critical period. When the ligature was applied at about 40 hours after pupation, the ectoderm differentiated, but in a certain percentage of these cases the development of the abdominal hairs (used as the criterion for differentiation) is not normal. As a consequence partly developed abdomens occur. One way of explaining these results by supposing the existence of a hormone is the assumption of an insufficient supply of hormone at the time of the operation.

In the course of our work on Drosophila, in which we used a combined transplantation and ligature technique, certain indications were found which suggested the rôle of oxygen in the process of imaginal differentiation. We may imagine that a Drosophila pupal abdomen lacking the whole anterior region is unable to obtain sufficient oxygen from the air to complete its development. As we know that in flies oxygen may be absorbed through the skin;[5] it was thought to be possible to bring about

development by putting such abdominal parts into a pure oxygen atmosphere. Such experiments were performed and gave, remarkably enough, positive results. As table 1 shows, abdomens, the anterior parts of which have been removed 40 hours after pupation, differentiate in oxygen completely in 100% of the cases, whereas the controls only show complete differentiation in 50% or less of the cases. Furthermore, pupae ligatured before the critical period, which in air would never differentiate at all could be made to differentiate completely in oxygen (up to 50% of the cases). If the critical period is an indicator of the time of action of a hormone center, pupae ligatured 21 hours after pupation, i.e., 7 hours before the critical period, could not have received any hormone. The differentiation of such abdomens in oxygen thus makes the assumption that these imaginal differentiation processes are controlled by a hormone very improbable.

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[3] Hachlow, V., *Ibid.*, **125,** S. 26 (1932).
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EXPERIMENTS ON THE RELATION OF NUTRITION TO THE COMPOSITION OF THE BODY AND THE LENGTH OF LIFE

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It has long been an influential view that the chemical composition of an organism is rather rigidly specific. Individual differences in quantity of inactive reserve stores, such as fat in the animal or starch in the plant, being taken as a matter of course, it has been held ever since Liebig that in its essential parts every normal individual will grow and develop to the specific chemical composition of its kind. And this doctrine which Liebig based on chemical analyses has its physiological corollary in the aphorism of Claude Bernard, that it is the *fixité* of the body's internal environment which enables it to cope with a new or changeable external environment. The neat form of this generalization, and its usefulness in helping to explain the self-regulatory processes in the body and the spread of species over the surface of the earth, have resulted in its having been accepted for two generations as almost axiomatic.

Meanwhile, however, there have developed physico-chemical views, based on much more complete evidence, which call for revision of these

over-simple postulates or first-approximations of Liebig and of Bernard. We can no longer doubt that when different proportions of active factors are introduced into a system some difference in concentration-levels or dynamic-equilibrium points must result.

Also, we now have experimental evidence that the outcome with respect to the quantitative chemical composition of the body is not parallel for all constituents. Thus it was strikingly shown by Osborne and Mendel that, with lysine as the limiting factor in the dietary, growth responds to the level of lysine intake in quite close approximation to the so-called "Liebig's law of the minimum"; but our present experiments with animals of the same strain show that restriction of the calcium content of an otherwise good diet results in animals growing up with calcium-poor bodies.

We now advance to the more ambitious and constructive aspect of this general problem. When we deal not with deficiencies but with differences within the range of the normal, how significantly does nutrition influence the chemical composition and internal environment of the body, its vitality or fitness and its length of life?

Both by calcium-balance experiments with children and by controlled experiments with animals whose entire bodies were directly analyzed for calcium at fixed ages, it has been established that the rate of calcification in the normally developing body, and therefore the percentage of calcium in the body at a given age or size, may be varied by adjustment of the nutritional intake of calcium at a higher or a lower normal level, and this without any immediately detectable departure from full health or from the accepted norms of physical development.

In our full-life and successive-generation experiments,[1] our Diet A (Laboratory No. 16) containing about 0.20 per cent of calcium in the dry food mixture has been found to approximate minimal adequacy for fully and permanently successful results generation after generation. This was therefore taken as the starting-point for the first series of experiments here reported. Parallel families of animals of the same strain were fed (*a*) on this Diet 16, (*b*) on the same enriched with calcium to 0.64 per cent and (*c*) on the same plus further enrichment with calcium to 0.80 per cent with simultaneous increase of phosphorus in the same proportion; and the young from these families were analyzed for body calcium at fixed ages. These experiments show that differences in the calcium content of the family food supply, within the range of normal experiences both in nature and in the present-day civilized life of human beings and their domestic animals, involve very significant differences in the calcium content of the body. The difference is not measurable at birth (at least in the average case) but develops rapidly with early growth, is largest at the time of greatest growth and is still significant in adult life (Fig. 1). Naturally the question arises whether the individuals receiving a more liberal

intake of calcium and thus making higher rates of retention and maintaining a higher percentage of body calcium at any given age, will show a higher level of nutritional well-being and general fitness throughout life.

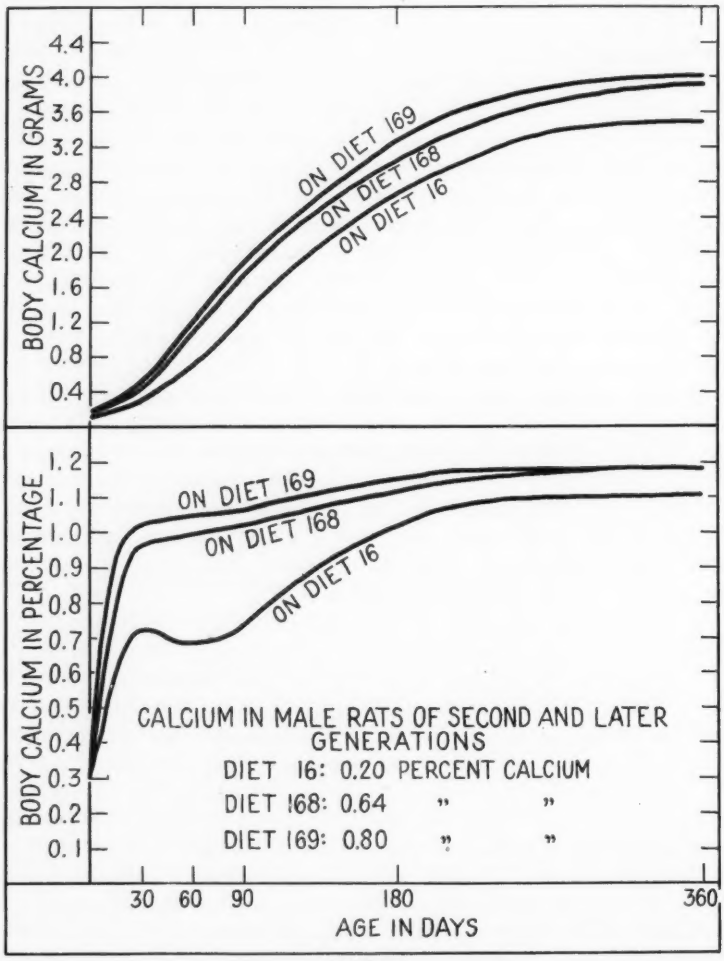

FIGURE 1
Amounts and percentages of calcium.

Many of the families on the diets just mentioned have now completed their natural lives; and the results thus far obtained are entirely favorable to the more liberal levels of calcium intake, and indicate that the higher

rate of retention here shown is a permanently advantageous expediting of a phase of the normal development of the growing body.

When the basal ration is our better-balanced Diet B (Laboratory No. 13) (which is half-again to twice as rich as Diet A (or 16) not only in calcium but also in vitamin A and riboflavin; and also improved in lesser degree in protein value), the increase of the calcium level from 0.35 to 0.48 or 0.64 per cent of the dry food mixture has no marked effect upon the rate of growth, but does (as in the previous series) increase the calcium content of the body. As these latter experiments are still in progress,

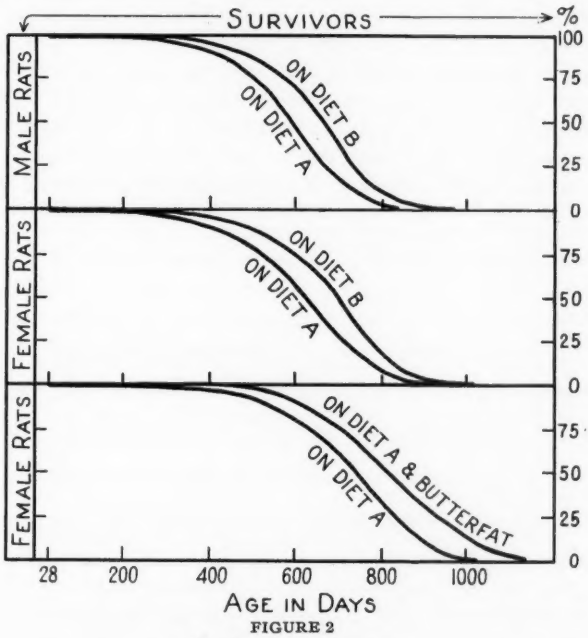

FIGURE 2

Survivorship diagrams.

the full-life records of nutritional well-being cannot yet be stated definitely.

The nutritional intake of vitamin A, adjusted to different levels by varying the proportion of butterfat in the dietary, has in our experiments significantly influenced the length of life and the vitality of the offspring, even when the rate of growth was not materially affected. This latter finding is of special interest as bridging a part of the apparent gap between the experiments of the Cornell[2] and Columbia laboratories upon the relation of nutritional régime to length of life. In our experiments it would

appear (Fig. 2) that the addition of extra butterfat alone to Diet A increased the length of life of these females to as large an extent as did Diet B which is richer not only in this respect but in calcium and riboflavin also. Two possible explanations suggest themselves, and both may perhaps be true: (1) It is conceivable that vitamin A may be somewhat specifically conducive to the lowering of death rates in the latter half of the life cycle as the survivorship diagram in figure 2 suggests. (2) It is also conceivable that the longer lives of the females receiving Diet A plus butterfat may be related to the fact that they did not, like those receiving Diet B, grow faster and bear and rear more young. We hope to study further the effects of different levels of intake of vitamin A.

As yet, each of our modifications of an already adequate dietary which has increased the length of life has extended the period of adult capacity and vitality rather than the period of physiological old age.

The coöperation of the Carnegie Corporation of New York and the Carnegie Institution of Washington is gratefully acknowledged.

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THE RESOLUTION OF TESTS INTO TWO GENERAL FACTORS

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In the Spearman[1] type of factor analysis, which was generalized by T. L. Kelley[2] to cover multiple factors and which is still of some interest despite the introduction of a different type of analysis by Hotelling, and of some supplementary considerations by Thurstone, there are conditions of the nature of inequalities for the existence of a solution. In the Spearman case for three tests it is necessary to find three quantities (communalities) h_1^2, h_2^2, h_3^2 such that the matrix

$$\begin{Vmatrix} h_1^2 & r_{12} & r_{13} \\ r_{12} & h_2^2 & r_{23} \\ r_{13} & r_{23} & h_3^2 \end{Vmatrix},$$

where the r's are the intercorrelations of the three tests, becomes of rank 1, i.e., such that all the 2×2 minors vanish. This requires in particular that

$$\begin{vmatrix} r_{12} & r_{13} \\ h_2^2 & r_{23} \end{vmatrix} = 0 \quad \text{or} \quad h_2^2 = \frac{r_{12}\, r_{23}}{r_{13}}.$$

Now h_2^2 is necessarily positive and between 0 and 1. Hence, with the similar results for h_1^2 and h_3^2, it appears that either 0 or 2 of the correlation coefficients must be negative. By reversal of one scale the case of 2 negative coefficients can be made one of 0, so that we may say that it shall be possible to take all 3 coefficients to be positive. This being done, the condition that the h's shall be positive proper fractions means that the partial correlations shall also be positive.[3]

If the tests be represented by unit vectors **a, b, c** the matrix may be written as[4]

$$\begin{Vmatrix} h_a^2 & \mathbf{a.b} & \mathbf{a.c} \\ \mathbf{a.b} & h_b^2 & \mathbf{b.c} \\ \mathbf{a.c} & \mathbf{b.c} & h_c^2 \end{Vmatrix}$$

where **a.b** is the scalar product of the vectors **a** and **b** and the conditions may be stated as that the angles between each pair of vectors shall be acute (or may be so taken by reversing one of the vectors) and that the angles between the three planes determined by pairs of the vectors shall also then be acute. Indeed if we write $h_b^2 = 1 - k_b^2$ and remember that $\mathbf{b.b} = 1$ the determinant becomes

$$\begin{vmatrix} \mathbf{a.b} & \mathbf{a.c} \\ 1 - k_b^2 & \mathbf{b.c} \end{vmatrix} = \begin{vmatrix} \mathbf{a.b} & \mathbf{a.c} \\ \mathbf{b.b} & \mathbf{b.c} \end{vmatrix} + \begin{vmatrix} 0 & \mathbf{a.c} \\ -k_b^2 & \mathbf{b.c} \end{vmatrix} = 0,$$

or, by a fundamental formula of multiple algebra,[5]

$$(\mathbf{a}\times\mathbf{b}).(\mathbf{b}\times\mathbf{c}) + k_b^2\, \mathbf{a.c} = -(\mathbf{b}\times\mathbf{a.b}\times\mathbf{c}) + k_b^2\, \mathbf{a.c} = 0$$

or

$$k_b^2 = \frac{\mathbf{b}\times\mathbf{a.b}\times\mathbf{c}}{\mathbf{a.c}} = \frac{\sin(\angle\ \mathbf{a, b})\, \sin(\angle\ \mathbf{b, c})\, \cos(\angle\ \mathbf{ab}\ \text{to}\ \mathbf{ac})}{\cos\ (\angle\ \mathbf{a.c})}$$

and $k_c^2 > 0$ means that the cosine of the angle between the planes **ab** and **bc** is positive.

Kelley discussed the case of the resolution of four tests into two factors in a good deal of detail.[6] When, however, he came to the resolution of five tests into two factors he obtained only one of the conditions[7]—his pentad equation. The analysis may be carried through in terms of vectors, not only to reach the pentad equation more neatly than Kelley did but also to indicate the inequalities which must be satisfied. We have a 5×5 matrix

$$\left\| \begin{array}{ccccc} 1-k_a^2 & \mathbf{a.b} & \mathbf{a.c} & \mathbf{a.d} & \mathbf{a.e} \\ \mathbf{a.b} & 1-k_b^2 & \mathbf{b.c} & \mathbf{b.d} & \mathbf{b.e} \\ \mathbf{a.c} & \mathbf{b.c} & 1-k_c^2 & \mathbf{c.d} & \mathbf{c.e} \\ \mathbf{a.d} & \mathbf{b.d} & \mathbf{c.d} & 1-k_d^2 & \mathbf{d.e} \\ \mathbf{a.e} & \mathbf{b.e} & \mathbf{c.e} & \mathbf{d.e} & 1-k_e^2 \end{array} \right\|$$

of which, for resolution into two tests all 3×3 minors must vanish. Of these one is

$$\left| \begin{array}{ccc} \mathbf{a.c} & \mathbf{a.d} & \mathbf{a.e} \\ \mathbf{b.c} & \mathbf{b.d} & \mathbf{b.e} \\ 1-k_c^2 & \mathbf{c.d} & \mathbf{c.e} \end{array} \right| = \left| \begin{array}{ccc} \mathbf{a.c} & \mathbf{a.d} & \mathbf{a.e} \\ \mathbf{b.c} & \mathbf{b.d} & \mathbf{b.e} \\ \mathbf{c.c} & \mathbf{c.d} & \mathbf{c.e} \end{array} \right| + \left| \begin{array}{ccc} 0 & \mathbf{a.d} & \mathbf{a.e} \\ 0 & \mathbf{b.d} & \mathbf{b.e} \\ -k_c^2 & \mathbf{c.d} & \mathbf{c.e} \end{array} \right|,$$

or

$$\mathbf{a}\times\mathbf{b}\times\mathbf{c.c}\times\mathbf{d}\times\mathbf{e} - k_c^2(\mathbf{a}\times\mathbf{b.d}\times\mathbf{e}) = \mathbf{c}\times\mathbf{a}\times\mathbf{b.c}\times\mathbf{d}\times\mathbf{e} - k_c^2(\mathbf{a}\times\mathbf{b.d}\times\mathbf{e}) = 0$$

and

$$k_c^2 = \frac{\mathbf{c}\times\mathbf{a}\times\mathbf{b.c}\times\mathbf{d}\times\mathbf{e}}{\mathbf{a}\times\mathbf{b.d}\times\mathbf{e}},$$

must be positive and between 0 and 1. This condition means a restriction of the nature of double inequality upon the five vectors **a, b, c, d, e** analogous to that arising in the case above—in fact, the vector form of k_c^2 here is a perfectly natural extension of the vector form given above. To turn the statement into geometric form would require a form of language adapted to 5-dimensional geometry.

In the formula for k_c^2 the vector **c** enters in a different manner from that of the other four vectors. Indeed it is clear that a number of such formulas may be written for k_c^2 merely by interchanging the rôles of **a, b, d, e** in the expansion. There are 24 such permutations but as the value of k_c^2 is not altered by interchange of **a** and **b** (both numerator and denominator interchanging sign) or by that of **d** and **e** or by the interchange of the pair **a** and **b** with the pair **d** and **e**, there are in fact only 3 different forms

$$k_c^2 = \frac{\mathbf{c}\times\mathbf{a}\times\mathbf{b.c}\times\mathbf{d}\times\mathbf{e}}{\mathbf{a}\times\mathbf{b.d}\times\mathbf{e}} = \frac{\mathbf{c}\times\mathbf{a}\times\mathbf{d.c}\times\mathbf{b}\times\mathbf{e}}{\mathbf{a}\times\mathbf{d.b}\times\mathbf{e}} = \frac{\mathbf{c}\times\mathbf{d}\times\mathbf{e.c}\times\mathbf{d}\times\mathbf{b}}{\mathbf{a}\times\mathbf{e.d}\times\mathbf{b}}.$$

This continued equality means that three identities of the form

$$(\mathbf{c}\times\mathbf{a}\times\mathbf{b.c}\times\mathbf{d}\times\mathbf{e})(\mathbf{a}\times\mathbf{d.b}\times\mathbf{e}) - (\mathbf{c}\times\mathbf{a}\times\mathbf{d.c}\times\mathbf{b}\times\mathbf{e})(\mathbf{a}\times\mathbf{b.d}\times\mathbf{e}) = 0$$

shall exist. This is, as a matter of fact, a vector form of the pentad equation, the other two forms being equivalent thereto although the expressions set equal to zero may be of opposite sign.

As **c** enters asymmetrically as compared with **a, b, d, e,** it must be that in solving for k_a^2, k_b^2, k_d^2, k_e^2 there will be other pentad equations such as

$$(\mathbf{a}\times\mathbf{b}\times\mathbf{c}.\mathbf{a}\times\mathbf{d}\times\mathbf{e})(\mathbf{c}\times\mathbf{d}.\mathbf{b}\times\mathbf{e}) - (\mathbf{a}\times\mathbf{c}\times\mathbf{d}.\mathbf{a}\times\mathbf{b}\times\mathbf{e})(\mathbf{b}\times\mathbf{c}.\mathbf{d}\times\mathbf{e}) = 0,$$

where **a** has interchanged rôles with **c.** The pentad expression is, however, skew symmetrical in the five vectors, as may be seen by reference to the expanded form in which Kelley writes it, so that any such new equations are in fact not new conditions upon the vectors or tests. Indeed by identifying two such expressions with the proper sign a large number of algebraic identities analogous to those found in books on three-dimensional vector algebra (or analogous to those found in ordinary spherical trigonometry) may be written down for the five dimensional case—for example:

$$(\mathbf{c}\times\mathbf{a}\times\mathbf{b}.\mathbf{c}\times\mathbf{d}\times\mathbf{e})(\mathbf{a}\times\mathbf{b}.\mathbf{b}\times\mathbf{e}) - (\mathbf{c}\times\mathbf{a}\times\mathbf{d}.\mathbf{c}\times\mathbf{b}\times\mathbf{e})(\mathbf{a}\times\mathbf{b}.\mathbf{d}\times\mathbf{e})$$
$$+ (\mathbf{a}\times\mathbf{c}\times\mathbf{d}.\mathbf{a}\times\mathbf{b}\times\mathbf{e})(\mathbf{b}\times\mathbf{c}.\mathbf{d}\times\mathbf{e}) - (\mathbf{a}\times\mathbf{b}\times\mathbf{c}.\mathbf{a}\times\mathbf{d}\times\mathbf{e})(\mathbf{c}\times\mathbf{d}.\mathbf{b}\times\mathbf{e}) = 0.$$

To work freely with the problem of resolving five or more tests into two general factors and the appropriate number of specifics it might be necessary to have a great deal of familiarity with such relationships.

If five vectors **a, b, c, d, e** be resolved into two generals and five specifics as

$$\mathbf{a} = r_{ag}\mathbf{g} + r_{ah}\mathbf{h} + \mathbf{s}_a, \qquad \mathbf{b} = r_{ag}\mathbf{g} + r_{ah}h + \mathbf{s}_b, \ldots$$
$$\mathbf{a}.\mathbf{b} = r_{ag}r_{bg} + r_{ah}r_{bh}, \qquad \mathbf{g}.\mathbf{s}_a = \mathbf{h}.\mathbf{s}_a = \mathbf{s}_a.\mathbf{s}_b = \ldots = 0,$$

it is clear that the continued (outer) product of the two generals **g, h** and any test **a,** viz., $\mathbf{g}\times\mathbf{h}\times\mathbf{a}$, will reduce to $\mathbf{g}\times\mathbf{h}\times\mathbf{s}_a$ which is perpendicular to the continued product $\mathbf{b}\times\mathbf{c}\times\mathbf{d}$ of any three tests other than **a,** since $\mathbf{s}_a$ is perpendicular to **b, c** and **d.** Hence for a resolution into two generals there must be a planar vector **M,** any multiple of $\mathbf{g}\times\mathbf{h}$, such that

$$\mathbf{M}\times\mathbf{a}.\mathbf{b}\times\mathbf{c}\times\mathbf{d} = \mathbf{M}\times\mathbf{a}.\mathbf{b}\times\mathbf{c}\times\mathbf{e} = \mathbf{M}\times\mathbf{b}.\mathbf{a}\times\mathbf{c}\times\mathbf{d} = \ldots 0, \qquad (1)$$

there being in fact 20 such equations. (In this equation **M** may be considered to be the projection $\mathbf{M}_0$ of **M** on the 5-space of the tests, and in what follows **M** will actually be considered to be this projection $\mathbf{M}_0$.) Is the converse true, that is, is it true that if there be a planar vector **M** such that all 20 of the equation (1) be satisfied then the five tests **a, b, c, d, e** are resoluble into two generals—provided that for a real resolution the necessary equalities, the form $0 \leqq k_a^2 \leqq 1$, are satisfied? The answer is: Yes. The proof is tedious but straightforward. One writes

$$\mathbf{M} = x_{12}\mathbf{a}'\times\mathbf{b}' + x_{13}\mathbf{a}'\times\mathbf{c}' + \ldots + x_{45}\mathbf{d}'\times\mathbf{e}'$$

where $\mathbf{a}', \mathbf{b}', \mathbf{c}', \mathbf{d}', \mathbf{e}'$ are the reciprocal system[5] to **a, b, c, d, e.** Then

$$\mathbf{M}\times\mathbf{a} = x_{12}\mathbf{a}'\times\mathbf{b}'\times\mathbf{a} + x_{13}\mathbf{a}'\times\mathbf{c}'\times\mathbf{a} + \ldots + x_{45}\mathbf{d}'\times\mathbf{e}'\times\mathbf{a}. \qquad (2)$$

The product $\mathbf{M}\times\mathbf{a.b}\times\mathbf{c}\times\mathbf{d}$ will yield only three terms, viz.,

$$\mathbf{M}\times\mathbf{a.b}\times\mathbf{c}\times\mathbf{d} = (\mathbf{a.d})x_{23} - (\mathbf{a.c})x_{24} + (\mathbf{a.b})x_{34}$$

and

$$\mathbf{M}\times\mathbf{e.b}\times\mathbf{c}\times\mathbf{d} = (\mathbf{d.e})x_{23} - (\mathbf{c.e})x_{24} + (\mathbf{b.e})x_{34},$$

from which the ratios $x_{23}:x_{24}:x_{34}$ may be obtained. The equations

$$\mathbf{M}\times\mathbf{a.b}\times\mathbf{c}\times\mathbf{d} = \mathbf{M}\times\mathbf{e.b}\times\mathbf{c}\times\mathbf{d} = \mathbf{M}\times\mathbf{c.a}\times\mathbf{b}\times\mathbf{e} = \mathbf{M}\times\mathbf{d.a}\times\mathbf{b}\times\mathbf{e} =$$
$$\mathbf{M}\times\mathbf{b.a}\times\mathbf{c}\times\mathbf{e} = \mathbf{M}\times\mathbf{d.a}\times\mathbf{c}\times\mathbf{e} = \mathbf{M}\times\mathbf{b.a}\times\mathbf{d}\times\mathbf{e} = \mathbf{M}\times\mathbf{c.a}\times\mathbf{d}\times\mathbf{e} =$$
$$\mathbf{M}\times\mathbf{c.b}\times\mathbf{d}\times\mathbf{e} = \mathbf{M}\times\mathbf{a.c}\times\mathbf{d}\times\mathbf{e} = 0$$

will suffice to determine[8] the ratios of all 10 coefficients x_{ij} and the condition that the pentad be zero comes out in the process as a relation between **a, b, c, d, e** necessary and sufficient for the solution to be obtained.

Now it was pointed out[4] that if the four or more vectors **a, b, c, d,** . . . were to be resoluble into one general and four specifics the spherical polygon defined by the termini of the vectors (supposed laid off from a common origin) must have an orthocenter or the polyhedral angle formed by the vectors must have an orthocentral line, and conversely if this holds and certain inequalities also hold the tests are thus resoluble. The theorem above affords the corresponding result for the resolution of five or more tests into two generals and five or more specifics: The spherical polygon defined by the termini of the vectors must have an orthocentral arc or the polyhedral angle must have an orthocentral plane,[9] which means that the pentads for every quintet of vectors must vanish, and conversely if these conditions hold and certain inequalities also hold the tests are thus resoluble.[10]

[1] C. Spearman, *The Abilities of Man.*

[2] T. L. Kelley, *Crossroads in the Mind of Man.*

[3] Thus if three tests have the intercorrelations 0.14, 0.25, 0.50, they may be resolved into one general and three specifics; but if they have the intercorrelations 0.14, 0.35, 0.50, they may not be so resolved. It is very doubtful whether sufficient attention has been paid by psychologists to the broad implication of these restrictive inequalities for the significance of a factor analysis.

[4] For notation see E. B. Wilson, *Proc. Nat. Acad. Sci.*, **14**, 283–291 (1928).

[5] See for instance A. N. Whitehead, *Universal Algebra*, **1** (p. 208), or E. B. Wilson, *Trans. Conn. Acad. Arts Sci.*, **14**, 1–57 (1908). Either of these references will also serve to define "reciprocal systems" referred to below. Notations and definitions may differ slightly though not essentially.

[6] More detail was supplied by Wilson and Worcester in *Proc. Nat. Acad. Sci.*, **20**, 189–192 (1934).

[7] Kelley, loc. cit., p. 58. See also E. B. Wilson, *Jour. Gen. Psychol.*, **2**, 153–172 (1929), footnote 3.

[8] The solution actually gives the values of the coefficients in (2) in terms of the original correlation coefficients r_{ij}. The coefficients are such that the plane **M** is a simple plane (not a complex one of the type $\mathbf{a}\times\mathbf{b}+\mathbf{c}\times\mathbf{d}$).

[9] A polyhedral angle of n edges, $n \geqq 5$, in n-space has an orthocentral plane when there is a plane through the vertex such that the 3-space formed by that plane and any edge l is perpendicular to all the 3-spaces formed from sets of three edges not including l (and hence perpendicular also to the $n - 1$ space formed by the $n - 1$ edges other than l).

[10] For the following two 5×5 matrices of intercorrelation coefficients

$$\begin{Vmatrix} \ldots & -.03 & .14 & .19 & .18 \\ -.03 & \ldots & .05 & .09 & .02 \\ .14 & .05 & \ldots & .08 & .04 \\ .19 & .09 & .08 & \ldots & .06 \\ .18 & .02 & .04 & .06 & \ldots \end{Vmatrix} \text{ and } \begin{Vmatrix} \ldots & -.045 & .135 & .18 & .18 \\ -.045 & \ldots & .05 & .09 & .02 \\ .135 & .05 & \ldots & .08 & .04 \\ .18 & .09 & .08 & \ldots & .06 \\ .18 & .02 & .04 & .06 & \ldots \end{Vmatrix}$$

the pentads vanish; the first is resoluble into 2 generals and 5 specifics, but the second is not (because some of the inequalities are not satisfied).

THE STEADY POLARITY POTENTIAL OF THE HUMAN EYE

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The existence of an electrical potential in the (freshly excised) eyeball of the frog has been recognized for some time. The corneal portion of the eye preparation is electrically positive, the retinal area negative. A relatively steady "current of rest," sometimes registering as high as 10 millivolts, is revealed when electrodes placed in contact with these two regions are connected to a sensitive galvanometer. In addition to this steady potential there occurs under the stimulation of light a small transitory "retinal action current." Excised eyes of many other species besides the frog have been studied with similar results.[1] Because excised preparations from warm-blooded animals soon become inactive, various attempts have been made and surgical techniques employed to make possible potential studies of mammalian eyes *in situ*.

Scientific interest has previously for the most part centered in the phenomenon of the eye's photo-electric response to light, and so while the action currents have been much studied, the phenomenon of the current of rest has been relatively neglected. Some valuable early and recent attempts to utilize the potential of the human eye in studies of nystagmus and other types of eye movement have been made. From these may be gleaned fragmentary information about the steady potential itself.

In the present paper a method is described for the direct study of the steady potential in the human eye *in situ* and some results and conclusions are reported. The following topics and materials are briefly considered:

the method used, without discomfort to the person examined, for the isolation of the ocular polarity potential; the circuit employed for recording and measuring eye potentials independent of loss in electro-motive force either from tissue resistance or from current drainage; the essential standard experimental conditions (bodily position and mental attitude) suitable for the human subject during the measurements; illustrative records together with evidence that the measured potential is derived from the bulbus and not from the recti muscles or other tissues associated with the eyeball; typical data from normal young subjects with tabulated results for different electrode leads; the simple mathematical relationships between different potential measurements from different standard lateral leads and the significance of these for a theory of the bio-electrical field of the eyes and immediately surrounding tissues.

The isolation of the steady potential of the eye, from the potentials of the skin and other surrounding tissues has been accomplished by a method of balancing the body potential and by using the eye's motility systematically to vary the recorded eye potential. The method is as follows: small metal foil electrodes covered with a thin layer of salt paste are placed on the skin lateral to the cornea on both the nasal and the temporal sides, and as near as possible to the eyes. They are held in position by bits of adhesive tape. The eye muscles can now perform the service of a revolving switch changing the contact relationships of the eyeball with the four fixed electrodes. The farther the eye is revolved to one side, the nearer the electrodes approximate contact with the anterior and posterior portions, and the stronger the potential indicated on the galvanometer. When the eye looks successively at a series of fixed points, for example, 10, 20, 30 and 40 degrees to the right or left of the primary line of regard, the photographed galvanometric deflections show as a series of regular and almost equal steps. The method of measurement here depends on the conduction of the eye potential to adjoining tissues. Theoretically this is not as desirable as direct electrode contact with the bulbus itself, but it is obviously not possible to introduce an electrode into direct contact with the posterior portion of the human eyeball, and contact with the corneal area usually produces real or imagined discomfort to most subjects. Fortunately there need be no discomfort involved in the attachment of the electrodes to the skin near the eyes, and the method is found to give results sufficiently reliable for an exploratory study. Furthermore in working by this method of potential isolation the highly accurate motor coördination of eye movement and fixation can be utilized advantageously. Guided by accurately placed fixation points a subject can turn his eye through a given visual angle more accurately than an experimenter can measure off a similar angle on his eyeball. The method of leading off by electrodes applied to the temples was followed by Meyer[2] and also by

Fenn and Hursh,[3] the former interpreting the recorded potentials as derived from the extra-ocular muscles.

In comparative study of ocular polarity-potentials the measurements should be essentially free from voltage drop produced by tissue resistance or current drainage. To insure this condition was the first aim of the preliminary stage in the development of the measurement technique. The result is the circuit shown in figure 1. Part III of the diagram shows the indispensable feature of the apparatus. Here a vacuum tube microvoltmeter of the Burr-Lane-Nims[4] type used as input to the Einthoven galvanometer (see Part IV), converts it in operation into an electrometer.*

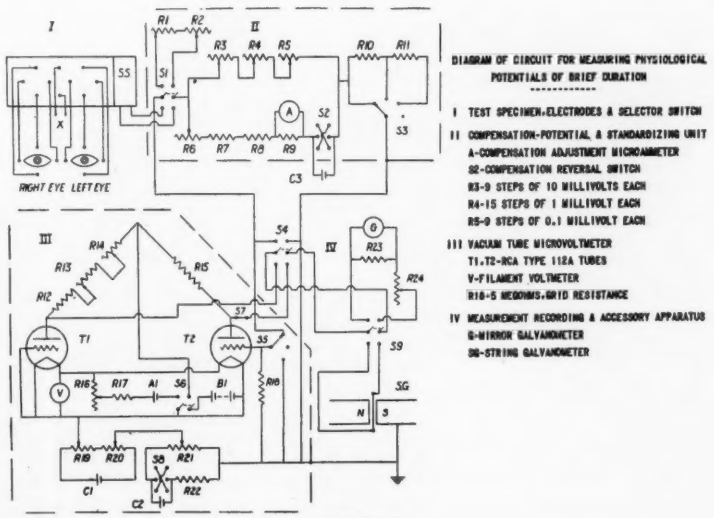
DIAGRAM OF CIRCUIT FOR MEASURING PHYSIOLOGICAL POTENTIALS OF BRIEF DURATION
I TEST SPECIMEN-ELECTRODES & SELECTOR SWITCH
II COMPENSATION-POTENTIAL & STANDARDIZING UNIT
A-COMPENSATION ADJUSTMENT MICROAMMETER
S2-COMPENSATION REVERSAL SWITCH
R3-9 STEPS OF 10 MILLIVOLTS EACH
R4-15 STEPS OF 1 MILLIVOLT EACH
R5-9 STEPS OF 0.1 MILLIVOLT EACH
III VACUUM TUBE MICROVOLTMETER
T1,T2-RCA TYPE 112A TUBES
V-FILAMENT VOLTMETER
R16-5 MEGOHMS,GRID RESISTANCE
IV MEASUREMENT RECORDING & ACCESSORY APPARATUS
G-MIRROR GALVANOMETER
SG-STRING GALVANOMETER
RIGHT EYE
LEFT EYE

FIGURE 1

Part II in the diagram shows a compensation-standardizing unit such as is commonly employed with the string galvanometer, when used as a recording instrument, as in the electrocardiograph. Body potential is compensated by this unit and standard potentials are introduced through the same unit for the sensitivity calibration of the galvanometer. Tissue resistance and current drainage are negligible by reason of the fact that one lead from the eyes connects directly to the grid, *T*2, and the other lead is taken to ground, while the grid leak, *R*18, of 5 megohms, completes the electrode circuit. Differences in the tissue resistance of different subjects, or of the same subject at different times, are prevented from importantly influencing the eye potential measurements by the use of the diagrammed circuit.

In making the measurements it is of primary importance that the co-operating individuals be comfortable and free from apprehension. Emotional activity causes rapidly changing skin potentials which may amount to several millivolts. Since the galvanometer has to be zeroized by compensation of the skin potential, before the eye deflections can be recorded, any rapid skin potential change introduces a rather serious complication in the process of recording. For the experiment the subject is seated facing the head rest and the system of fixation lights, figure 2, with his back to the measuring equipment, in a comfortable upright chair, adjustable as to height. He rests his feet on the floor or on a footstool, his

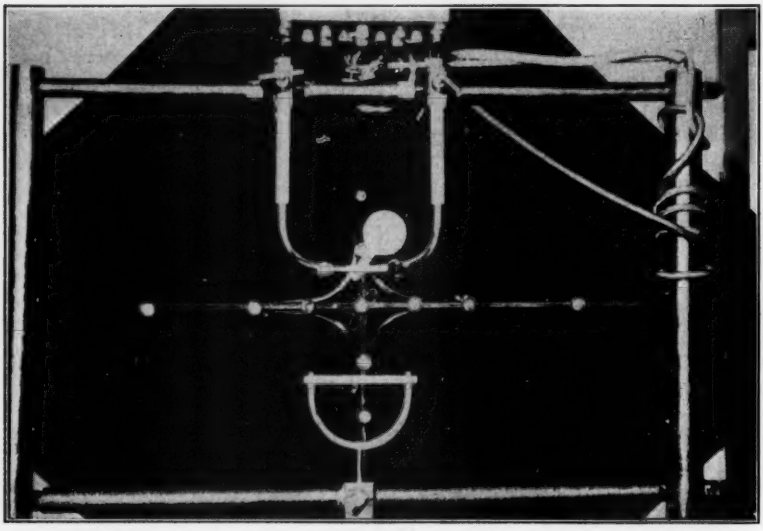

FIGURE 2

hands are in his lap. The room is rather dimly lighted by the projection lamp for the galvanometer and the neon fixation lights. The head rest, figure 2, is highly adjustable, permits a clear view over a wide field and is not cramping or otherwise unpleasant to the subject. In the illustration the supports for the forehead and the mouth (a bit of clean wood replaced for each subject) are shown in the foreground; the jacks into which the electrode connections are inserted appear at the top. The fixation points seen in the background are small neon lights accurately placed on horizontal and vertical axes, each of which has a radius of curvature of 150 cm. measured from the mid-point between the patient's eyes. From the central fixation light, *C*, successive lights subtend 5, 10, 20, 30 and 40 degrees of visual angle. The neon lights are sufficiently visible so that

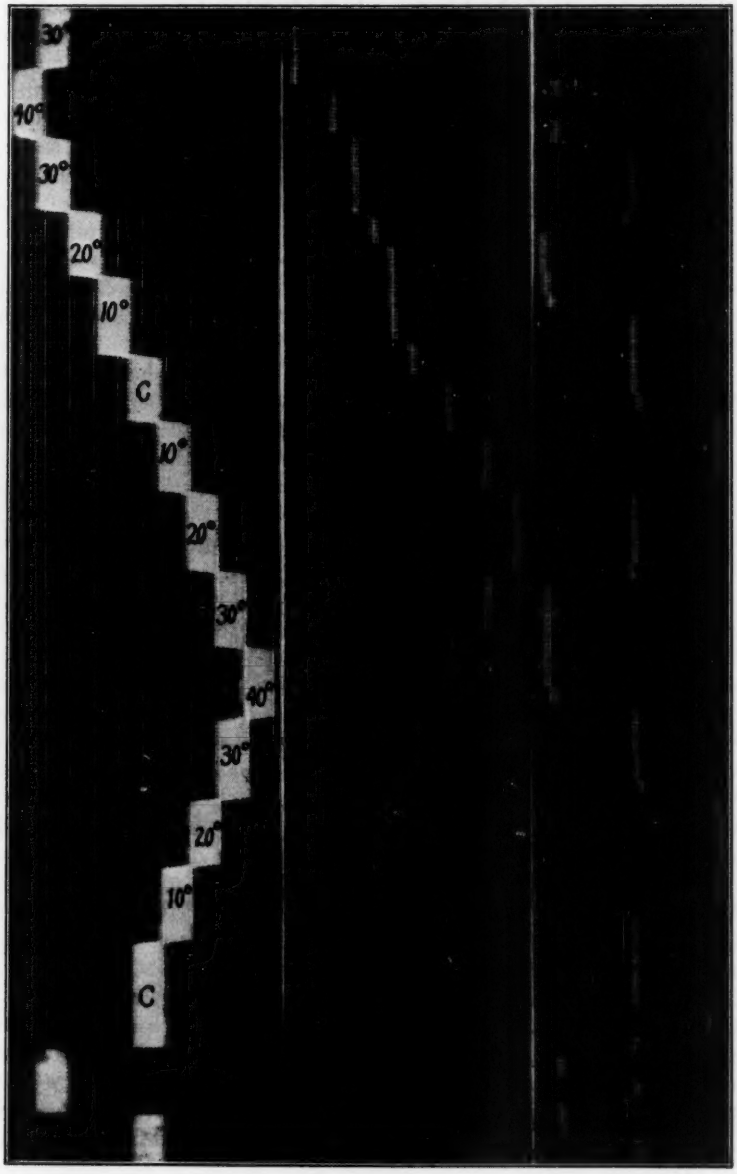

FIGURE 3

the measurements of all subjects can be made without the aid of spectacles. Each subject is repeatedly instructed to hold his head still. The experimenter who is taking the record stands back of the subject, directing him by verbal instructions, and designating the lights which are to be fixated. When a subject's ability to coöperate with the instructions is limited or doubtful, an assistant standing in front of him and to one side verifies the subject's fixations by direct observation. According to the subject's rate of adjustment one or more seconds are allowed for each fixation in order that the eye may be properly directed and then held in place for a period long enough to make a strip of record that is measurable. Each record includes a standard deflection from a 0.001 volt source preceding the series of eye potential deflections, and the latter are measured in terms of this standard deflection.

The routine measurement of ocular polarity-potentials has been simplified for this report by using only the center and the 30 degree fixation lights. Since my results agree with those of Fenn and Hursh[3] in giving values for the potential that appear to be proportional to the sine of the visual angle, it seems probable that the potential values for 30 degree rotations approximate half of what might be expected if the eye could be rotated 90 degrees to a point at right angles to the primary line of regard. The eye potential value for 30 degree rotations is used in the present research as the standard measurement for each subject. Further discussion reported here is limited to measurements and results from lateral eye rotation records.

Three sample records are reproduced in figure 3. They all show rotations of the eye to the right and left and are to be read upward from bottom to top. The heavier white vertical lines in the background mark 5 mm. distances (amplitudes), and the heavier white horizontal lines 0.2 sec. durations. The magnification is 1000 times. Record A was taken with a string of 0.007 mm. diameter and 510 ohms resistance. The record is of a young man, F. L. C., whose eye movements were very rapid and precise. The electrodes were placed on the temples lateral to and next the eyes. The initial 20 mm. deflection to the left was made from a 1.0 millivolt standard source for the purpose of standardizing the sensitivity of the instrument. Starting with fixation on the center light, *C*, the lights 10, 20, 30 and 40 degrees to the right of *C* were successively viewed. Then the eyes were shifted from 40 back to 30 and so by successive steps until they reached *C* again; from here they were shifted in similar steps to the left and back again. The record is cut short in the reproduction after the 30 degree fixation when returning to center from the left. The general proportionality of the potential to the angle of eye rotation is evident; 30 degrees in this case equals about 1 mv. Record B was taken, with the more usual string of 0.002 mm. diameter and 6500 ohms resistance,

of a young woman, A. A., whose fixations were prompt and careful. Here also the electrodes were placed on the temples lateral to and just next the eyes. After the standard deflections, from 1 mv. applied twice to the left and once to the right, visual fixation of the center light, *C*, was followed by fixations of the lights 5, 10, 20, 30 and 40 degrees to the right and then in reverse order, extending to 20 degrees to the left of *C*, where the illustrative record terminates. This subject shows 20 degrees of lateral rotation equal to 1 mv. Record C is also from subject A. A., but with the lead-off from the left eye only. This is a routine polarity-potential record (lateral) using only the center and 30 degree fixation points. The small notches which occur at the beginning of most of the fixations represent corrective movements of the eye (often required when the eye takes a large angle in one continuous sweep), and are marks of precise care in fixation. The measurements from which potentials are computed are, of course, made from those portions of the record which do not include the correction notches. Standard deflections from 1 mv. are shown at the beginning and end of the record.

Illustrative records A and B in figure 3 substantiate the finding that the eye potential is not influenced by the direction of rotation over a given arc in the visual field, either away from the primary line of regard or toward it. For example, if the eye moves from the central point, *C*, to the right 10 degrees and then back to *C*, the two potentials registered in these deflections will usually equal each other within the error of measurement. On the other hand equal angles of rotation on opposite sides of *C* do not always register as equal potentials especially when the lead-off is from one eye only. Obviously the placement of the electrodes is a factor of importance and it is not feasible to attach them in perfectly symmetrical relationships about the two bulbi as measured separately. Furthermore the distribution of the potential on the bulbus is probably not uniform and possibly not symmetrical. These various factors cannot be discussed in detail here.

Several lines of evidence point to the conclusion that the measured potential is derived from the bulbus and not from the recti muscles or other tissues associated with the eyeball. First, if we were dealing with action current phenomena from the extra-ocular muscles we should expect our records to show spike or wave-like deflections (similar to electrocardiograms) rather than the relatively square notches here exhibited. Winks do record as spikes. The electrode placement is favorable for leading-off action currents from the lid muscle (obicularus) but not from the recti. Second, the speed of deflection in the potential records agrees with the angular velocity rates found by photographing the eye (corneal reflection method) during the execution of saccadic eye movements, the type used in reading. In records A and B the series of deflections to the right from

C closely resemble reading records such as are taken photographically in examining reading habits. Third, from a study in this research of several cases of unilateral enucleation it has been found that even though a glass eye has considerable motility the polarity-potential is practically absent. If not entirely absent it is minimal as compared with the potential of the active eye, and from its direction appears to be merely a spread from the latter. Fourth, measurements made with the electrodes placed lightly but directly against the sclera of the eyeball (in voluntary subjects) show, for 30 degree eye rotations, larger potentials and in the same direction (polarity) as found with electrodes on the skin just next the eye. Finally,

TABLE 1

OCULAR POLARITY-POTENTIALS IN 15 NORMAL SUBJECTS MEASURED FROM LATERAL EYE ROTATIONS OF 30 DEGREES*

NO.	AGE	SEX	DE†	LD. 1 −LE−	LD. 2 −RE−	LD. 3 −LE RE−	LD. 4 LE—RE	LD. 5 −RE—LE−
100	8	F	L	0.89	0.83	1.21	0.52	1.00
32	10	F	L	0.68	0.74	0.80	0.49	0.66
108	12	M	R	0.82	0.88	1.29	0.50	0.86
98	12	F	L	0.41	0.67	0.71	0.47	0.60
45	12	M	L	0.76	0.80	1.19	0.46	0.55
111	14	M	L	0.57	0.47	0.78	0.32	0.62
80	18	M	L	0.87	0.63	0.88	0.31	0.63
122	18	M	R	0.57	0.53	0.77	0.31	0.59
123	20	M	R	0.58	0.78	0.91	0.41	0.66
2	21	F	L	0.98	1.13	0.97	0.92	0.87
1	21	M	R	0.68	0.59	0.95	0.34	0.80
26	21	M	R	0.92	1.07	1.52	0.69	1.09
130	22	M	R	0.71	0.76	1.03	0.46	0.67
28	23	M	R	0.94	0.85	1.08	0.64	0.73
54	25	F	L	0.93	1.22	1.36	0.51	1.10
			Av.	0.75	0.80	1.03	0.49	0.76
			A. D.	0.14	0.16	0.20	0.11	0.15

* Values in millivolts.

† Visual acuity 20/20 or better in all 15 subjects.

the many studies made of freshly excised eye preparations of animals show quite definitely that the living eyeball has a steady potential when examined independent of its motor equipment. Most convincing of these studies for our present purpose is one made in this Laboratory by Mowrer, Ruch and Miller[5] in which the passive eye of an anesthetized animal when moved was found to show regular potential changes. Furthermore when the recti had been detached from the eye, the potential changes continued to appear on passive movement. Typical normal data now follow with a brief discussion.

Normal children, adolescents and young adults, measured by the method outlined, show an average polarity-potential of about 0.8 mv. for each

eye examined separately when the eyes are rotated laterally 30 degrees from the primary line (see table 1). Each value entered in the table is an average in millivolts of rotations to the right and rotations to the left of the center point, *C*. For Lead 1 the electrodes were placed on the skin as near as possible on either side of the left eye; for Lead 2, a similar arrangement was made for the right eye. Lead 3 makes use of the outside (temporal) electrodes on each eye; Lead 4 employs the inside (nasal) electrodes. In Lead 5 the left temporal and right nasal electrodes are joined and taken to one galvanometer terminal, while the left nasal and right temporal electrodes are joined and taken to the other terminal. In other words, the two eyes are connected in a parallel system. From table 1 it will be seen that a pair of eyes may give almost equal potentials or may differ considerably from each other. Seven of the cases show the two eyes within 10 per cent of each other, 4 exhibit differences of about 25 per cent; one of these, No. 98, shows the left eye with a measured potential 39 per cent lower than the right. It is not clear that the dominant eye for sighting (DE) is regularly associated with high or low potentials; 7 of the 15 cases presented show the dominant eye with the higher potential. The range for single eyes exhibited in the table is from 0.41 to 1.22 mv.; other data not shown here reveal cases both higher and lower than these. No obvious correlation of eye potential with age or sex appears in this sample; it is clear that a much larger population will be required for the drawing of any correlational conclusions.

By examining the interrelationships between ocular polarity-potential measurements made with different electrode leads we may expect to learn something concerning the bio-electrical field state of the eyes and their immediately surrounding tissues. The present sample of data appears to be sufficiently homogeneous and representative to serve this purpose. Examination of table 1 shows that use of the two temporal electrodes, Lead 3, ordinarily (there is but one clear exception, No. 2) results in a higher potential measurement than either eye demonstrates when measured by itself. Although both eyes are involved in the temporal lead, it should not, I think, be understood as giving the series potential of the two eyes, but rather a value approaching their optimal mean. It is evident that Lead 3 is not the sum of Leads 1 and 2, but represents an increase of about $^1/_3$ over their mean. The result may be due to the more favorable lead-off position of the temples as compared to the nasal areas. It should be stated that frequently when eyes are measured separately the right shows a higher value for 30 degree rotations to the right than to the left, and conversely for the left eye. For purposes of simplification in the present treatment, right and left rotations have been averaged for each eye as for the other leads. But temporal leads are not the equivalent of nasal leads. It will be noted that Lead 4 shows an average potential about

$^1/_2$ that of Lead 3 and $^2/_3$ the average of Leads 1 and 2. Lead 5, a parallel connection involving all four lateral electrodes, gives an average potential closely equal to the mean of Leads 1 and 2.

The general relationships which appear to be typical between the lateral measurement leads and the ascertained ocular polarity-potentials may be stated as follows:

$$\text{Ld.1} + \text{Ld.2} = \text{Ld.3} + \text{Ld.4}. \qquad (1)$$

The averages in table 1 fit this formulation to within 2 per cent. Among the 15 cases for whom data are given, 9 deviate from the formula by less than 6 per cent. There is one, No. 80, who shows Ld.3 + Ld.4 as 20.6 per cent less than Ld.1 + Ld.2. An average of the deviations from (1) of each of the 15 subjects gives 6.6 per cent.

$$\text{Ld.5} = {}^1/_2(\text{Ld.1} + \text{Ld.2}). \qquad (2)$$

Lead 5 amounts to placing the eyes in a parallel connection. It will be seen from the table that (2) holds closely for the general averages; the obtained value is 49 per cent. Individuals deviate between (No. 45) 35.3 and (No. 1) 63.0 per cent, while half the cases fall between 45 and 55, and the average of the 15 individual results is 49.7 per cent.

$$\text{Ld.5} = {}^1/_2(\text{Ld.3} + \text{Ld.4}). \qquad (3)$$

The Ld.5 average of 0.76 mv. (table 1) is just 50 per cent of average 1.03 + average 0.49 mv. Again it is found that cases No. 45 and No. 1 deviate most markedly showing 33.3 and 62 per cent, respectively. Ten of the subjects fall between 45 and 55 per cent, and the average for the 15 individual cases is 50.5 per cent.

These results are what we might expect if we regard the eyeballs as actively and continuously polarized and if we think of them and their surrounding tissues as a conducting dielectric system or interconnected group of such systems. The electrically negative, deeply embedded, posterior portions of the eyes may be assumed to contribute a negative charge to the neighboring deep-lying tissues. The positive anterior portions of the bulbi are in large part exposed between the eyelids and have by means of them and the conjunctiva, contact with the skin and superficial tissues. Rotation of the eyes produces potential changes at the points of contact of the electrodes on the skin. Whether the electrodes are influenced equally and oppositely by the negative and positive areas of the eyeball or primarily by the changes in the position of the positive area cannot be definitely answered at present. In either case the relationships found between the lateral measurement leads indicate that under conditions of minimal current drainage the deeper tissues surrounding and including the eyeballs, while probably made negative largely through the

electrical characteristics of the bulbi, all act as conductors or as an electrical field of essentially uniform character.

Conclusions.—1. A steady potential from the eyeball can be isolated for measurement from the potentials of the skin and surrounding tissue by making use of the eye's motility.

2. The measurable ocular polarity-potential of the human eye *in situ* is in the order of 1 millivolt when the eye rotates laterally 30 degrees from the primary line of regard; the range found between supposedly normal subjects is from about 0.3 to 2.5 millivolts.

3. The potential can be measured, without discomfort to the subject, by means of small metal foil electrodes placed on the skin near the eye, and connected to a vacuum tube microvoltmeter used as input for a string galvanometer. In this way the measurement is freed from the complication of voltage drop due to tissue resistance and current drainage since the instrument acts as an electrometer.

4. The potential exhibited by the eyeball is closely proportional to the sine of the angle of rotation of the eye, and therefore is to prove useful as a means of measuring eye movements in connection with a variety of scientific problems.[6]

5. Galvanometric deflections registering the steady polarity-potential of the eye show no evidence of action currents from the extraocular muscles, but do frequently show action current spikes from voluntary and reflex winking.

6. Subjects who have suffered enucleation of one eye show only a very slight potential around the glass eye, and this potential appears to be a spread from the active eye. This finding indicates that the results from measuring each eye separately when both are in place and active are probably not greatly in error through mutual electrical influence.

7. The two eyes do not register equal potentials in most cases thus far examined. The potentials of the eyes can be measured in parallel but not in series in the same person. The potential of one eye may be made to oppose that of the other eye and partly to neutralize it.

8. When both eyes are active electrodes placed on the temples ordinarily give a higher potential than do those from any other placement on the skin.

9. Data reported from a study of 15 young normal subjects show the presence of certain simple relationships existing between the lateral measurement leads and the ascertained potentials. These relationships may be reduced to simple equations indicating equivalence of certain combinations of leads. The results support the hypothesis that the eyeballs and their orbital and interorbital tissues act, at least under conditions of minimal current drainage, as one homogeneous bioelectrical field.

10. The practical medical value of the measurement of ocular polarity-

potential is wholly problematical at present. There is some hint that the potential varies in certain diseases, but this and other phases of the problem remain to be further investigated.

11. The cause and significance of polarity-potentials is the chief problem under investigation here. The eyes are unique not in having steady electromotive force, probably a characteristic of body tissues generally, but rather in having rapid and accurate motility which makes the isolation and measurement of their potentials practicable.

* For a fuller discussion of the performance of the string galvanometer with input through a vacuum tube microvoltmeter see W. R. Miles, *Jour. Exptl. Psychol.* (in press 1939).

[1] A. Kohlrausch, "Elektrische Erscheinungen am Auge," Bethe's *Handbuch der Normalen und Pathologischen Physiologie*, Bd. 12, Text 2, p. 1396, Springer, Berlin, 1931; also W. S. Duke-Elder, *Text-Book of Ophthalmology*, p. 835, Mosby, St. Louis, 1934.

[2] I. L. Meyer, *Arch. Neurol. and Psychiat.*, **21**, 901 (1929).

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THE PHYSICAL STATE OF THE INTERSTELLAR GAS CLOUDS

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1. The investigations by Eddington[1] and by Gerasimovič and Struve[2] suggested that the state of ionization of the interstellar gas may be very high. In fact, it has usually been considered probable that nearly all Ca atoms are doubly ionized, and that only a small fraction—perhaps $^1/_{1000}$—are singly ionized. Similarly, for Na the computations gave a ratio of $^1/_{300,000}$ for Na/Na^+. This result of the computations was in marked disagreement with the observed fact that the interstellar absorption lines of Ca II are somewhat weaker than those of Na I. Only the assumption of an unreasonably high abundance of Na—of the order of 300 atoms of Na to one atom of Ca—could explain the strength of the Na lines.

An attempt by Eddington[3] to explain this discrepancy as a result of the curve of growth computed for rotational Doppler effect[4] has not met with success.[5]

An even more drastic contradiction between theory and observation has become apparent through the recent discovery by Dunham[6] of interstellar absorption lines of Ti II, Ca I and K I. Evidently, the degree of ionization

of the interstellar gas is much lower than the early computations had suggested. It is, therefore, necessary to revise the theory.

2. Dunham's[6] discovery of the interstellar resonance line of Ca I, λ 4226.73, is particularly valuable. Although he considers it possible that "there is one chance in several hundred that the observed line is not due to Ca I," the agreement in wave-length is almost perfect, and the cumulative evidence of the lines of low ionization potential of Ti II and K I is so convincing that it seems best to rely upon the identification and to use it for a preliminary computation of the degree of ionization.

The line of Ca I is very weak. Although Dunham has not yet given the measured intensities, these will, in any case, be rather uncertain because of the extreme weakness of the line. The weak interstellar lines lie on the steep straight line branch of the curve of growth.[7] Dunham has observed Ca I in χ^2 Orionis and 55 Cygni. In these stars the equivalent width of Ca K is of the order of 0.5 A. Assuming that the Ca lines fall on a curve of growth which is similar to the one derived by Wilson and Merrill[8] for Na, and that the equivalent width of the Ca I line is of the order of 0.01 A, we find approximately:

$$\frac{N_2(Ca^+)}{N_1(Ca)} \approx 100 \text{ or } 150. \qquad (1)$$

This value is somewhat uncertain, but it is sufficient for a rough orientation. It is certainly very much better than anything available in our former work. In order to use the ionization equation we must know T (the temperature of the radiation, which we shall assume here to be equal to the electron temperature T_e) and β (the dilution factor). For the reaction $Ca \longleftrightarrow Ca^+$ we are concerned with the spectral region near λ 2000. The energy curves of the stars may be extrapolated with a reasonable degree of certainty from the observed violet limit, and we shall adopt here approximately the values derived by Gerasimovič and Struve,[2] $T = 15{,}000°$, and $\beta = 10^{-16}$. They agree reasonably well with a more recent derivation of the energy density of stellar radiation in space derived by Greenstein.[9]

If we substitute (1) in the equation of ionization:

$$\log \frac{N_2}{N_1} Ne = -\chi_1 \frac{5040}{T} + 1.5 \log T + 15.38 + \log \frac{2u_2}{u_1} \qquad (2)$$

where $\chi_1 = 6.1$ volts, is the first ionization potential of Ca, and if we disregard the statistical weights and the partition functions—the latter because practically all atoms are in the ground level—we have, roughly:

$$\log N_e = 17.5 \text{ (for thermodynamic equilibrium).}$$

For interstellar space, we have, accordingly,

$$\log N_{e(space)} = 17.5 - \log \beta = 1.5.$$

This corresponds to about 30 free electrons per cm.^{3}—a value which seems rather large, but which results directly from (1), provided that (2) may be applied in the sense suggested by Eddington[1] and that, moreover, $T = T_e$. We shall adopt this value for $N_{e(space)}$ and use it to determine the reactions $Na \longleftrightarrow Na^+$ and of $Ca^+ \longleftrightarrow Ca^{++}$. For the former we take $\chi_1 = 5.1$ volts and this is so close to the value used for Ca, that we shall again adopt $T = 15{,}000$, $\beta = 10^{-16}$. The result is, with our former value of $N_{e(space)}$:

$$\frac{N_2(Na^+)}{N_1(Na)} = 270. \qquad (3)$$

Since the ionization potential of Ca^+ is 12 volts, we are now dealing with the region near λ 1000 A, and we can no longer use the same values of T and β. It is evident, on general grounds, that T will be higher and β smaller. A reasonable compromise between the data of Greenstein and those of Gerasimovič and Struve is $T = 25{,}000°$, $\beta = 10^{-17}$. Using again our value of $\log N_{e(space)} = 1.5$, we obtain:

$$\frac{N_3\,(Ca^{++})}{N_2\,(Ca^+)} = 12. \qquad (4)$$

3. The values of $N_1(Na)$ and $N_2(Ca^+)$ are known from the observations of the equivalent widths of the interstellar lines. We adopt for Na the value by Wilson and Merrill:[8]

$$N_1(Na) = 3 \times 10^{-9} \text{ per cm.}^3, \qquad (5)$$

and for Ca II by Merrill and Sanford:[10]

$$N_2(Ca^+) = 9 \times 10^{-9} \text{ per cm.}^3, \qquad (6)$$

The second ionization of Na can be neglected, because of the high ionization potential. The same is true of the third ionization of Ca. Hence, we can use (1), (4) and (5) to compute the total number of Ca atoms and (3) and (5) to compute the total number of Na atoms. The result is

$$N_1(Na) + N_2(Na) = 8 \times 10^{-7} \text{ per cm.}^3. \qquad (7)$$

$$N_1(Ca) + N_2(Ca) + N_3(Ca) = 1.2 \times 10^{-7} \text{ per cm.}^3. \qquad (8)$$

The ratio is about 7 to 1, which compares not unfavorably with the terrestrial abundance ratio of about 1.3 in favor of Na. The striking anomaly of the former computations has disappeared. The reason for this improvement lies in two points: (*a*) we have computed the electron density

$N_{e(space)}$ from the reaction $Ca \longleftrightarrow Ca^+$ and (*b*) we have used different sets of T and β for the reactions near 1000 A and near 2000 A. The second point is especially important. The result depends, principally, upon the decrease of β by a factor of 10 in passing from λ 2000 A to λ 1000 A. The exact numerical value of β is unimportant and affects only $N_{e(space)}$, but not $N_{e(thermodynamic\ equilibrium)}$. Accordingly, all ionizations remain exactly the same if β (2000 A)/β (1000 A) = 10 and if the temperatures remain 15,000° and 25,000°, respectively. However, if β is increased at λ 1000, $N_{e(space)}$ will be increased in proportion and this would require too much H. If both values of β can be reduced, say, by a factor of 10 the agreement between $N_{e(space)}$ and the probable amount of available H will be nearly perfect. But we have at present no excuse for such an arbitrary change in β.

4. We shall next consider the origin of $N_{e(space)}$. Obviously, Ca^+, Ca^{++} and Na^+ can contribute only an insignificant fraction of the required number of free electrons. In this respect our present theory departs radically from that of Gerasimovič and Struve[11] where all elements were assumed to be equally effective in the production of free electrons, so that N_e could be determined from the equation

$$pN_e = N_2(Ca^+) + 2N_3(Ca^{++}),$$

where $p = 0.015$ is the percentage abundance of Ca by atoms in the crust of the earth. This equation led to a very small value of N_e (about 10^{-3}), which in turn accounted for the high degree of ionization.

In order to justify our larger value of $N_{e(space)} = 30$, we must show that there is a reasonable supply of free electrons. The most promising source is interstellar H, the existence of which—reasonable on general grounds—has recently been suggested by spectrographic observations at the McDonald Observatory.[12]

Elvey and I have shown from the observations of 35 regions in the Milky Way that about 22 show the H_α line in emission. Many of these regions show also H_β and [O II] 3727 but only a very few show N_1 and N_2. The bright lines of H_α and [O II] 3727 are apparently not related to any particular bright O or B stars: they are usually seen over the entire length of the slit—about 1°.5—and show no pronounced tendency to become stronger in the vicinity of definite stars. For example, in the large star clouds of Cygnus and Cepheus the emission is present over very large areas of the sky, covering hundreds of square degrees. If it forms a single, nearby nebula it must be related to a very bright star, such as γ Cygni or α Cygni. This is not at all probable, because there is little increase in nebular brightness as we approach these stars. Rather are we inclined to believe that we are dealing with interstellar clouds of gas which are excited

to emission by the ultra-violet radiation of many hot stars in the star clouds of the Milky Way. The distinction between the two types of nebulae is, at best, rather arbitrary. It is an observational fact that the radiation of very hot stars, which is perhaps appreciably reinforced by diffuse nebular emission in the Lyman continuum, is sufficient to produce bright H lines at very great distances. This conclusion is supported by the observations of Greenstein and Henyey[13] and those of Struve and Elvey[14] who find that the outermost portions of the Orion nebula, at distances of at least 20 parsecs from the exciting stars, show strong emission lines of H and [O II]. It is quite probable that in ordinary interstellar matter the ultra-violet radiation of the stars is still effective at distances of 100 or more parsecs.[15]

Our hypothesis of the interstellar origin of the extended fields of H emission receives strong support from the fact that these fields are apparently very abundant in the constellations of Cepheus and Cygnus, where the interstellar absorption lines are abnormally strong. On the other hand, the Orion region, which is strong in H emission, gives relatively weak interstellar absorption lines. I attribute this to the fact that in the Orion region the entire excitation comes from relatively close, very hot stars of types O and early B. The temperature should, therefore, be roughly constant for all wave-lengths (provided, of course, we can rely upon the λ^{-1} law of interstellar reddening to hold throughout) approximating a value of about 25,000°. The dilution constant will also be approximately the same for all wave-lengths, being a function of the distance from the exciting stars. At about 10 parsecs it will be of the order of 10^{-15}. If the electron density is the same as in interstellar space, the ionization will be greatly increased, both for $Ca \longleftrightarrow Ca^{+}$ and for $Ca^{+} \longleftrightarrow Ca^{++}$. The lines of Ca II should, therefore, be fainter. But an increase in N_e will compensate for the increase in β, and it is difficult to predict the outcome.

I have determined elsewhere[12] that for interstellar space the number of atoms of H in the third quantum level, in a cylinder whose cross-section is 1 cm.^{2}, is

$$D.N_1^{(3)} = 10. \tag{9}$$

If we assume that this cylinder has, on the average, a length D = 1000 parsecs, we find

$$N_1^{(3)} = 3 \times 10^{-21} \text{ per cm.}^3. \tag{10}$$

Since the third level of H is not metastable we can use the approximate relation[16]

$$\frac{N_1^{(3)}}{N_1^{(1)}} = \frac{g_3}{g_1} \beta e^{-h\nu/kT}. \tag{11}$$

We are here again dealing with the region around 1000 A and shall, therefore, use $T = 25{,}000°$, $\beta = 10^{-17}$. The result is

$$N^1(H) = 3 \times 10^{-2} \text{ per cm.}^3. \tag{12}$$

We consider next the ionization of H. Using again $T = 25{,}000°$, $\beta = 10^{-17}$, $\log N_{e(space)} = 1.5$, we find

$$\frac{N_2 (H^+)}{N_1 (H)} = 6. \tag{13}$$

Accordingly, H would give us 0.2 free electrons per cm.^{3}. This is insufficient by a factor of about 100. The discrepancy, although large, appears by no means hopeless. Our expression (11) is extremely crude and may require a large correction.[16] Our original value in (1) can hardly be in error by as much as a factor of 10. But the assumption $T = T_e$ is probably not correct. There are several effects to be considered: (*a*) the electrons are here supposed to come from H and will hence have a higher temperature than the particular T which is appropriate for the reaction $Ca \longleftrightarrow Ca^+$; (*b*) T_e may be raised even higher, if the energy curves of the stars present strong Lyman continuous absorptions; (*c*) T_e will have a tendency to decrease because of collisions prior to the processes of recombinations. It is not possible at present to estimate these effects, and it is, therefore, fruitless to use the more complicated ionization equation[17] which allows for differences between T and T_e. The values of T and β at 1000 A may be very different from those which we have used. Greenstein's computation allows for the effect of interstellar reddening, according to the λ^{-1} law. But it is, of course, quite uncertain whether we can treat the energy curves of the stars as black bodies. The only available evidence rests upon the success of Zanstra's theory in the interpretation of the planetary nebulae.

A simple change in β does not improve the situation. If we reduce β we can get more H atoms in the ground state. But this will at the same time reduce the degree of ionization of H, and hence will not appreciably change the number of free electrons available from H.

Dr. Kuiper has called my attention to the fact that Oort's dynamical limit allows a maximum of about 4 atoms of H per cm.^{3}. This is ample to account for the observed emission lines of H, but it suggests that our electron density $N_{e(space)}$ may be too large. Since this resulted directly from the reaction $Ca \longleftrightarrow Ca^+$ it is well to consider it in detail. It is not likely that the electron temperature can be low enough to reduce N_e by a factor of 10. It is very improbable that β is much smaller than 10^{-16} or that T is far from 15,000°. There may be an appreciable error in (1) although hardly by as much as 10. At the present moment it seems most likely that several factors combine to exaggerate $N_{e(space)}$.

The discrepancy is not very serious, especially when we consider that the

absorption lines of Ca I, Ca II, Na I, etc., are produced in regions of interstellar space where the ionizing radiation is relatively weak, while the emission lines of H (and of [O II]) come from regions where there is relatively more radiation in the region of 1000 A. Perhaps we should rather be surprised that after having passed from the excessively small number of H atoms in the third energy level to the very large number in the ground level, we come within a factor of about 100 to the value of N_e computed from interstellar absorption lines.

5. Perhaps the most significant result of this investigation is the unexpectedly high abundance of H. We find, roughly, 10^6 for the ratio of H to Ca, by atoms. Approximately the same value holds for the ratio of H to Na.

Since practically the entire mass of the interstellar gas is made up of H atoms, the density is approximately

$$\rho = 3 \times 10^{-25} \text{ gr./cm.}^3.$$

This result agrees remarkably well with Eddington's original estimate based upon dynamical and thermodynamical considerations.[18]

The great intensity of the H emission lines in the Orion nebula and the corresponding faintness of the Na and Ca II absorption lines can be explained principally as the effect of β in (11). For interstellar space we used $\beta = 10^{-17}$. For a representative point in the Orion nebula $\beta = 10^{-15}$. Hence, if all other factors are the same, the bright Balmer lines will be 100 times stronger in the nebula than in interstellar space. The state of ionization of the nebula will depend upon β/N_e. But the ratio of $N_1(H)/N_2(Ca^+)$ does not change much when we pass from interstellar space to the Orion nebula.

[1] *The Internal Constitution of the Stars*, German Edition, 478 (1928).

[2] *Ap. Jour.*, **69**, 19 (1929).

[3] *M. N.*, **95**, 2 (1934).

[4] Unsöld, Struve and Elvey, *Zeit. Ap.*, **1**, 314 (1930).

[5] C. S. Beals, *M. N.*, **96**, 661 (1936).

[6] *Pub. A. S. P.*, **49**, 26 (1937); *Nature* **139**, 246 (1937).

[7] Wilson and Merrill, *Ap. Jour.*, **86**, 51 (1937). It should be remembered that for the strong line of Ca II the curve of growth is quite flat. Hence estimates of the ratio $N_2(Ca^+)/N_1(Ca)$ are necessarily very uncertain. The only available curve of growth is constructed by means of stars of different distances, and it is not at present possible to correct this curve for the effect of distance. I am inclined to believe that the error should not be greater than by a factor of 10, and for our present discussion this is sufficient.

[8] *Ap. Jour.*, **86**, 67 (1937).

[9] Unpublished. Greenstein allows for interstellar reddening according to λ^{-1} but neglects scattered radiation. His data give at λ 2000: $\beta = 7 \times 10^{-16}$ if $T = 15{,}000°$; and at λ1000: $\beta = 4 \times 10^{-17}$ if $T = 25{,}000°$. Gerasimovič and Struve find at λ 2000: $\beta = 1.5 \times 10^{-16}$, $T = 12{,}600°$; and at λ 1000: $\beta = 2.2 \times 10^{-17}$, $T = 15{,}300°$. The difference between the two sets may arise, in part, from the manner in which the stars have been lumped.

[10] *Ap. Jour.*, **87,** 122 (1938).
[11] *Ap. Jour.*, **69,** 20 (1929).
[12] Struve and Elvey, *Ap. Jour.*, **88,** 364 (1938).
[13] *Ap. Jour.*, **87,** 79 (1938).
[14] *Ap. Jour.*, **87,** 559 (1938).
[15] It should, however, be noted in this connection that Eddington (*M. N.*, **96,** 6 (1934)) has expressed the opinion that "in a normal region of interstellar space the hydrogen will be entirely un-ionized, and indeed in molecular form." However, this conclusion rests upon "the photons of energy greater than 13.5 volts . . . . . having been absorbed and transmuted by the hydrogen near the stars."
[16] It would be interesting to improve this relation by a numerical computation similar to that which was carried out for He I by Struve and Wurm, *Ap. Jour.*, **88,** 93 (1938).
[17] Gerasimovič, *Proc. Am. Acad. Arts Sc.*, **62,** 164 (1927); Harvard Reprint No. 38.
[18] *The Internal Constitution of the Stars*, German Edition, p. 467 (1928).

GROUPS OF DEGREE n IN WHICH THE LARGEST DEGREE OF A SUBSTITUTION IS A MINIMUM

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It was proved recently in these PROCEEDINGS, **24,** 202–204 (1938) that a necessary and sufficient condition that there is a group of degree n whose substitution of largest degree is as small as $n/2 + 1$ is that n is of the form $2^m - 2$, $m > 1$, and that when this condition is satisfied there is one and only one such group of degree n. Each of the other substitutions of this group, besides the identity, is also of degree $n/2 + 1$ and the group is abelian and of type 1^{m-1}. In a later article published in these PROCEEDINGS, **24,** 293–296 (1938), the possible groups of degree n in which the substitution of largest degree is either of degree $n/2 + 3/2$ or of degree $n/2 + 2$ were also considered. It was proved that there are three infinite systems of such groups in addition to a few special cases. In the present article we shall consider the more general question of finding groups of an arbitrary degree whose substitution of largest degree is relatively small.

When n is an arbitrary even number it results from the theorem noted above that there is a substitution group of degree n whose substitution of largest degree may be found as follows: If n exceeds 4 diminish n by the largest possible number which is of the form $2^\alpha - 2$ and repeat the operation, if necessary, until the remainder is one of the following three numbers 0, 2 or 4. If the number of these operations is k when the remainder is 0 a group of degree n can be constructed according to the theorem noted at the opening of the preceding paragraph whose substitution of largest degree is of degree $n/2 + k$. If the remainder after these k operations is

2 the largest degree of a substitution in this group is $n/2 + k + 1$. Finally, when this remainder is 4 after these k operations the degree of this substitution of largest degree is $n/2 + k + 2$. For instance, when $n = 100$ we reduce n and the remainders successively by 62, 30 and 6 and hence the group constructed according to the given theorem has for its substitution of largest degree a substitution of degree 54. Each of the transitive constituents of the group thus constructed is of degree 2. In particular, the group is abelian.

When n is odd it necessarily exceeds unity. We may then proceed similarly by reducing n and the successive remainders by the largest possible numbers of the form $2^m - 2$, $m > 2$, which give positive remainders. If after k such operations the remainder does not exceed 5 we can construct according to the given theorem a group of degree n whose substitution of largest degree is of degree $n/2 + k + 3/2$. It should be noted that while there is only one group of a given degree when the largest degree of a substitution is $n/2 + 1$ there are different groups of degree n in which the largest degree of a substitution exceeds this number. In a few of these cases the possible groups were determined in the second article cited in the first paragraph of the present article. It was noted there that the degree of the group is not necessarily completely determined by the degree of the largest substitution when this degree is not $n/2 + 1$. In particular, when this degree is $n/2 + 2$ the degree of the group may be either of the form 2^m or of the form $2^m - 4$.

A second method for studying this question is to determine the groups of largest degree which involve a given substitution s as their substitution of largest degree. When s is of order 2 we may reduce the degree of s successively by the largest powers of 2 which give positive remainders and then determine the largest degrees of the substitution groups which involve a substitution of largest degree which is such a power of 2. For instance, when s of degree $50 = 32 + 16 + 2$ it may be noted that the largest degree of a group which contains a substitution of degree 32 as its substitution of largest degree is of degree $32 + 16 + 8 + 4 + 2 = 62$. The largest degree of a group which contains a substitution of degree 16 as its substitution of largest degree is $16 + 8 + 4 + 2 = 30$. Since all the substitutions, except the identity, in these groups are of the same degree and there is only one group which has a substitution of degree 2 as its substitution of largest degree it results that the largest degree of a group which contains the given s as its substitution of largest degree is 94. The smallest possible order of such a group is 32 and the largest possible order is $32 \cdot 16 \cdot 2 = 1024$.

A necessary and sufficient condition that the group is completely determined by s when s is of order 2 is that the degree of s is a power of 2. In this case the order of the group is equal to this degree of s and all of

its substitutions besides the identity are also of this degree and of order 2. In all other cases the smallest order of the possible groups is equal to the largest power of 2 which is less than the degree of s while the largest order is equal to the product of the powers of 2 obtained by first finding the largest power of 2 which is less than the degree of s and then finding the largest power of 2 which is not greater than the remainder obtained by subtracting from the degree of s the given power of 2, etc. In all cases the groups are abelian since all of their substitutions are of order 2. The degrees of the resulting groups are always equal to the sum of the degrees of the constituent groups since all the substitutions in these constituent groups besides the identity are of the same degree. If k is the number of the different powers of 2 which are obtained by first reducing the degree of s by the largest power of 2 which does not exceed the degree of s and then reducing the remaining degree by the largest power of 2 which does not exceed the degree of this remainder, etc., and if these powers of 2 are 2^{α_1}, 2^{α_2}, etc., then the largest degree of the groups which involve s as their substitution of largest degree is equal to the sum of $2^{\alpha_1} - 2$, $2^{\alpha_2} - 2$, etc. In order to determine for any given even degree the substitution of largest degree such that the degree of this substitution is as small as possible it is only necessary to reduce this even degree by the largest number of the form $2^{\alpha} - 2$ which gives either a positive remainder or 0 for a remainder and then proceed with this remainder, if it exceeds 0, in a similar way. The degree of required substitution is then the sum of the given numbers of the form $2^{\alpha-1} + 1$ by which the degrees are reduced.

The preceding method, with slight modifications, can be used to determine a substitution of smallest degree in the possible groups of a given odd degree which has the property that it is the substitution of largest degree in at least one of these groups. When this odd degree is 3 such a substitution is of degree 3 and when this degree is 5 such a substitution is of degree 4, as was stated above. It may be noted that *if* s *is a substitution of smallest degree in the groups of a given odd degree* n *which has the property that it is of largest degree in at least one of these groups then the order of* s *is even except in the case when* $n = 3$. A proof of this theorem results from the fact that if the order of s is odd and the degree in question is odd the degree of s cannot be n, for if it would be n it would then be possible to construct a dihedral group of degree n in which the largest degree of a substitution would be less than n. In fact, such a dihedral substitution group could also be constructed if the degree of the group would exceed the odd number n since a cycle of arbitrary odd order could then be extended by transpositions so as to obtain a substitution of degree n and the group generated by it, less one transposition, could be extended so as to obtain the required dihedral group.

If $n > 4$ it is always possible to construct substitution groups of degree n

such that the largest degree of a substitution contained therein is $n - r$ where r exceeds 0. It follows from what is stated above that n can be so selected that r is an arbitrarily large positive integer. When r has a given value for the number n it does not necessarily have as large a value for the possible groups of degree $n + 1$, but when the degree of these groups exceeds $n + 1$ it is possible to find groups such that r has at least as large a value therein as it has for a possible group of degree n since the largest degree of a group in a direct product is the sum of the degrees of its constituent groups. For instance, when $n = 6$ the value of $r = 2$ as may be seen from the groups of order 4 which involve only positive substitutions of order 2 besides the identity, but when $n = 7$ it is not possible to find a group in which r exceeds unity. When $n = 8$ the largest value of r is obviously again equal to 2.

In the groups noted above which have the property that they separately involve a substitution of smallest degree for the possible groups of that degree such that this substitution is of largest degree in at least one group of that degree all the transitive constituents of this group were of degree 2 or 3. To see that such a transitive constituent may be of as large a degree as 6 it may be noted that the dihedral group of order 12 obtained by extending the regular cyclic group of order 6 may be made isomorphic with the intransitive group of degree and of order 4 so as to obtain a group of degree 10 and of order 12 in which there are ten substitutions of degree 8. It is easily seen that 8 is the smallest degree in a substitution group of degree 10 which has the property that it is a substitution of largest degree in the group in which it appears. What precedes leaves various questions unanswered in regard to the minimum degree of the substitution of largest degree in the groups of an arbitrary degree, but it is hoped that it exhibits progress along this line.

EULER'S CONCORDANT FORMS

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1. Numerous diophantine equations, or systems of such equations, in the literature are amenable to what was called the method of reciprocal arrays.[5] Even when this method does not furnish a complete solution, it frequently reduces the central difficulty to classical problems which have been at least partly solved. Such is the case, for example, with what Euler[1] in 1780 called concordant forms. A complete theory of these

forms is reducible to a very special case of the problem of making a binary quartic a square. It is interesting to observe that substantially this special case appears incidentally in Euler's own discussion of the general binary quartic problem.[2]

2. The forms $x^2 + ay^2$, $x^2 + by^2$ are *concordant* if the system

$$x^2 + ay^2 = u^2, \quad x^2 + by^2 = v^2, \tag{1}$$

in which a, b are constant integers and $ab(a - b) \neq 0$, has at least one non-trivial solution in integers x, y, u, v. The excluded possibilities for a, b offer no difficulty. All letters shall denote integers.

Let g be any common divisor of a, b, and let a_1a_2, b_1b_2 be any resolutions of a/g, b/g into two factors,

$$a = ga_1a_2, \quad b = gb_1b_2; \quad k \equiv a_1a_2 - b_1b_2. \tag{2}$$

For each set g, a_1, a_2, b_1, b_2 there will be an equation (3) and its corresponding set of formulas (4).

If σ, ϕ, ψ are such that

$$g\sigma^2 = \phi\psi(a_2\phi - b_1\psi)(a_1\psi - b_2\phi), \tag{3}$$

then (1) has the solution given by

$$\begin{aligned} 2kx &= \theta(a_2b_2\phi^2 - a_1b_1\psi^2), \\ 2ku &= \theta(2a_1a_2\phi\psi - a_1b_1\psi^2 - a_2b_2\phi^2), \\ 2kv &= \theta(2b_1b_2\phi\psi - a_1b_1\psi^2 - a_2b_2\phi^2), \\ ky &= \theta\sigma. \end{aligned} \tag{4}$$

The solution (4) appertains to a particular (3), which in turn appertains to a particular (2). For a particular (2), the corresponding (3) may have no solution σ, ϕ, ψ; also, no equation (3) may have a solution, in which case (1) has no solution. All solutions, if any, of (1) for fixed a, b are given by (4); moreover, it suffices to take g the greatest common divisor of a, b.

A necessary and sufficient condition that (1) be solvable is that at least one equation (3) be solvable. If $N(n)$ is the number of decompositions of n into a product of two factors, there are $N(a/g)N(b/g)$ equations (3), and hence at most this number of sets (4). If a particular (3) is solvable, it suffices to take $\theta = 2k\tau$, τ an integer, to get a solution of (1); all solutions appertaining to a particular solution of (3) are obtainable in an obvious manner by examining the residues modulo $2k$, or k, of the right-hand members of (4).

The rational solutions of $z^2 = Q(x, y)$, $Q(x, y) \equiv$ a binary quartic, have been discussed by several writers,[3,4] who have shown how to deduce chains of solutions from any particular solution; but the problem of determining a primitive solution has not been solved generally.

3. Conversely, (3) enables us to find a, b such that (1) has solutions. For example, the general solution of $\sigma^2 = \phi\psi$ is $\sigma = \alpha\beta\gamma$, $\phi = \alpha\beta^2$, $\psi = \alpha\gamma^2$, where β, γ may be taken coprime. Hence, if $\sigma^2 = \phi\psi$ in (3), we get

$$g = \alpha^2(a_2\beta^2 - b_1\gamma^2)(a_1\gamma^2 - b_2\beta^2).$$

Thus if in (1),

$$a \equiv a_1a_2\alpha^2(a_2\beta^2 - b_1\gamma^2)(a_1\gamma^2 - b_2\beta^2),$$
$$b \equiv b_1b_2\alpha^2(a_2\beta^2 - b_1\gamma^2)(a_1\gamma^2 - b_2\beta^2),$$

a solution of the equations is given by

$$2kx = \theta\alpha^2(a_2b_2\beta^4 - a_1b_1\gamma^4),$$
$$2ku = \theta\alpha^2(2a_1a_2\beta^2\gamma^2 - a_1b_1\gamma^4 - a_2b_2\beta^4),$$
$$2kv = \theta\alpha^2(2b_1b_2\beta^2\gamma^2 - a_1b_1\gamma^4 - a_2b_2\beta^4),$$
$$ky = \theta\alpha\beta\gamma,$$

with k as in (2).

As a second example, the choice $\phi = \psi = 1$ in (3) gives

$$g\sigma^2 = (a_1 - b_2)(a_2 - b_1),$$

which, by the method cited,[5] has the complete solution

$$g = g_1g_2,\ \sigma = \alpha\beta\gamma,\ a_2 = b_1 + g_1\alpha\beta^2,\ b_2 = a_1 - g_2\alpha\gamma^2,$$

with g_1, g_2, a_1, b_1, α, β, γ arbitrary integers. Hence

$$a \equiv g_1g_2a_1(b_1 + g_1\alpha\beta^2),\ b \equiv g_1g_2b_1(a_1 - g_2\alpha\gamma^2)$$

give a solvable system (1). Since any integer a may be written as the product of four integers, say $a = pqrs$, it follows that the infinity of forms $x^2 + by^2$, where

$$b \equiv pq(s - p\alpha\beta^2)(r - q\alpha\gamma^2),$$

and α, β, γ are arbitrary integers, are concordant with any given form $x^2 + ay^2$.

[1] The history of the subject is given by L. E. Dickson, *History of the Theory of Numbers*, **2**, 472–477 (1920).

[2] E. Haentzschel, *Jour. für die r.u.a Mathematik*, **144**, 277, Equation (8) (1914).

[3] History in Dickson, loc. cit., 639–644.

[4] L. J. Mordell, *Quarterly Jour. Math.*, **45**, 178–181 (1913–1914). Also Haentzschel, loc. cit.

[5] E. T. Bell, *American Jour. Math.*, **55**, 50–66 (1933).

ON THE MAPPING OF ABSTRACT SPACES ON POLYTOPES

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The first theorems, due to Alexandroff,[1] on the continuous mapping of a compactum on a polytope have been variously extended notably by the present writer[2] and later also by Kuratowski,[3] but always to separable metric spaces. Extensions to normal spaces under sufficient, if more or less restricted, conditions have proved not too remote. Our present object, however, is to outline a theorem giving *necessary and sufficient conditions* for the existence of the mapping.

The basic space $\mathfrak{R} = \{x\}$ shall be a $\mathfrak{B}$-space, i.e., an open set space, the open sets being merely subjected to the structural axioms: 0, $\mathfrak{R}$, the sum of any number of open sets, and the intersection of any two open sets, are all open sets. *No separation axioms are imposed.*

Let $\mathfrak{V} = \{U_i\}$ be a point-finite open covering of $\mathfrak{R}$ (each point x is on a finite number of sets of $\mathfrak{V}$). Let Φ be a geometric nerve of $\mathfrak{V}$ (deduced from the abstract nerve by "filling" in the simplexes with points by means of barycentric coördinates). The topology of the geometric complex Φ is fixed by taking as basis for the open sets on Φ the stars in all the derived $\Phi^{(n)}$ of Φ.[4]

We shall say that an open covering $\mathfrak{V}'$ is a derived of $\mathfrak{V}$ whenever its sets may be labelled $U'_{i \ldots j}$ under the following two conditions:

(*a*) There is at most one $U'_{i \ldots j}$ for each intersection $U_i \ldots U_j \neq 0$ and $U'_{i \ldots j} \subset U_i \ldots U_j$;

(*b*) The only non-void intersections in $\mathfrak{V}'$ are of the form: $U'_{i \ldots j} \ldots U'_{i \ldots j \ldots k}$.

From (*a*) follows that $\mathfrak{V}'$ is point-finite, and from (*b*) that we may choose for geometric nerve of $\mathfrak{V}'$ a subcomplex Φ_1 of Φ'. If there exists a *derived sequence* $\mathfrak{V}^0 = \mathfrak{V}, \mathfrak{V}', \ldots$ where $\mathfrak{V}^{(n+1)}$ is a derived of $\mathfrak{V}^{(n)}$, the covering $\mathfrak{V}$ is called *analytical.*

A continuous mapping T: $\mathfrak{R} \longrightarrow \Phi$ shall be called *canonical relative to* $\mathfrak{V}$ whenever it maps the strict intersection of any group of sets $U_i, \ldots, U_j$ of $\mathfrak{V}$ (i.e., the set of all points on these but on no other sets of $\mathfrak{V}$) onto the simplex representing the intersection $U_i \ldots U_j$ in the nerve Φ. Suffice it to say that the mappings of the Alexandroff type are all canonical. And now we have

THEOREM 1. *The n. a. s. c. for the existence of a mapping canonical relative to* $\mathfrak{V}$ *is that* $\mathfrak{V}$ *be analytical. Moreover all the mappings of* $\mathfrak{R}$ *on a polytope arise in this manner.*

Let T be called *quasi-canonical relative to* $\mathfrak{V}$ whenever it is as before except that Φ is topologized by its Euclidean metric. Then

THEOREM 2. *When* T *is quasi-canonical relative to* $\mathfrak{V}$, *then* $\mathfrak{V}$ *is analytical.*

Let $\mathfrak{V}$ be called *neighborhood-finite*[5] (= n. f.) whenever every point has a neighborhood which meets at most a finite number of sets of U.

THEOREM 3. *When* $\mathfrak{R}$ *is normal and* $\mathfrak{V}$ *is n. f. there exists a mapping of* $\mathfrak{R}$ *which is quasi-canonical relative to* $\mathfrak{V}$.

THEOREM 4. *For a* $\mathfrak{V}$*-space normality* $\cong$ *every binary open covering is analytical.*

In Theorem 4 "binary" could be replaced by "finite" or "n. f." Since non-normal spaces are known to exist, there exist spaces with non-analytical finite open coverings.

If dimension is defined by means of the orders of finite open coverings, we can also prove the complementary dimensional mapping result of Alexandroff for normal spaces. For a general $\mathfrak{V}$-space the same result may be obtained for the "analytical" dimension, i.e., defined by means of analytical coverings alone.

Generalization. Let $\mathfrak{V}'$ be related to $\mathfrak{V}$ as follows: Its sets are labelled $U^1_{i\ldots j}$, where the set just written $\subset U_i \ldots U_j$, and the intersections in $\mathfrak{V}'$ are in accordance with (b) when superscripts are disregarded. $\mathfrak{V}'$ is called a *generalized* derived of $\mathfrak{V}$. Suppose that we have a generalized derived sequence $\mathfrak{V}^0 = \mathfrak{V}, \mathfrak{V}', \ldots$, with nerves $\Phi_0 = \Phi, \Phi_1, \ldots$. There exists a projection $\Theta_n : \Phi_{n+1} \longrightarrow \Phi'_n$, which for $n = 0$ is described by $U^1_{i\ldots j} \longrightarrow$ the centroid of the simplex representing $U_i \ldots U_j$ in Φ. We have then

THEOREM 5. *Under the circumstances just described there exist canonical mappings:* T_n: $\mathfrak{R} \longrightarrow \Phi_n$ *such that* $\Theta_n T_{n+1} = T_n$.

From this it is but a step to the imbedding theorem of Menger-Nöbeling, along the lines of a proof which we have given some years ago. Theorem 5 also plays an essential rôle in proving the central proposition which we have given in a recent Note.[6]

[1] P. S. Alexandroff, *Ann. Math.*, **30**, 101–187 (1928).

[2] S. Lefschetz, *Ann. Math.*, **35**, 118–139 (1934).

[3] C. Kuratowski, *Fundam. Math.*, **24**, 259–268 (1935).

[4] Let Φ^* denote the complex topologized by a metric Euclidean relative to the barycentric coördinates. Then Φ, Φ^* are topologically equivalent when and only when all the stars are finite dimensional. J. W. Tukey pointed out to us the very interesting fact that Φ is likewise metrizable, but in a metric which is unrelated to the metric of Φ^*. See the Note immediately following this by J. W. Tukey.

[5] This type of covering has been introduced for a different purpose by A. W. Tucker (Princeton lectures, spring 1937).

[6] These PROCEEDINGS, **24**, 392–393 (1938).

THE INTRINSIC METRIC OF A POLYTOPE

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In the preceding note, Lefschetz has used a new topology for polytopes. It is the purpose of this note to describe an interesting metric giving this topology. This metric is defined in a purely combinatorial manner. A polytope in the new metric is a uniformly locally contractible space. The abstract application of this construction leads to the construction of an n-cell from $n + 1$ vertices.

1. We consider a polytope, P, which is a point set described by barycentric coördinates in terms of the vertices of the abstract simplicial complex, K. The successive regular subdivisions of K are: $K = K^{(0)}$, $K^{(1)}$, $K^{(2)}$, ..., and their vertices are points of P.

A sequence of $n + 1$ vertices, $b = a_0, a_1, \ldots, a_n = c$, joins b to c over $K^{(p)}$ if the simplexes $a_k a_{k-1}$, $k = 1, 2, \ldots, n$, belong to $K^{(p)}$. $\Delta^{(p)}(b,c)$ is the least value of n for which this is possible. If b and c are vertices of $K^{(p)}$ and hence of $K^{(p+1)}$, a simple combinatorial argument shows that $\Delta^{(p+1)}(b, c) = 2\Delta^{(p)}(b,c)$. We define the distance $\rho(b,c)$ of any pair of vertices of any $K^{(p)}$ by $\rho(b, c) = \text{min.}[1, 2^{-p}\,\Delta^{(p)}(b, c)]$. The uniqueness is ensured by the result above.

If x is any point of P, and $a^{(p)}$ is a vertex of that simplex of $K^{(p)}$ on which x lies, then $\{a^{(p)}\}$ is a Cauchy sequence in this metric. Hence it is easily seen that the metric may be extended by continuity to all the points of P.

Since the open sphere about $a^{(p)}$ of radius 2^{-p} is the star of $a^{(p)}$ in $K^{(p)}$, it is easy to show that this metric gives the topology used by Lefschetz.

2. As may easily be seen, every set of P of diameter less than $2^{-(p+1)}$ lies on the star of some $a^{(p)}$ in $K^{(p)}$, so that we have the

THEOREM: *Every set of* P *of diameter less than* $2^{-(p-1)}$ *can be deformed into a point over a set of diameter less than* $2^{-(p-1)}$.

This result is false for polytopes of unbounded dimension in the Euclidean metric.

3. We observe that the process of defining the distance $\rho(b,c)$ of vertices b and c of $K^{(p)}$ can equally well be carried out for the vertices of a sequence of abstract regular subdivisions of an abstract simplicial complex K. If we take K to consist of a single n-simplex and its faces, we obtain in this way a metric space with a countable number of points. If we complete this space in its metric, it is easy to see that we obtain a space homeomorphic to an n-simplex.

ON THE CONCEPT OF A TOPOLOGICAL SPACE

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In the following note, we shall generalize the notion of a topological space in such a way that it will be possible to distinguish between the bounded and the unbounded portions of a space. There are many topological problems in which it is essential to be able to make this distinction. For example, if we wish to develop a completely satisfactory theory of connectivity, we must be able to tell whether or not a cycle lies in a bounded portion of the space (cf. the remarks below).

The space of classical topology can be defined in terms of the primitive concepts of *point* and *C-set* (closed set). We shall find it preferable, however, to replace the concept of a *C*-set by that of a *CB-set* (closed, bounded set). Thus, a *topological space* S will consist of a class of undefined elements P_i, called the *points* of S, together with a class of point sets B_j, called the *CB-sets* of S, subject to the following three conditions:

(i) The empty set must be a *CB*-set.

(ii) The union of two *CB*-sets must be a *CB*-set.

(iii) The intersection of an arbitrary set of *CB*-sets must be a *CB*-set.

The *C*-sets of S will now be expressible in terms of the *CB*-sets in the following manner. A point set of S will be a *C-set* if, and only if, its intersection with every *CB*-set is a *CB*-set. Clearly the empty set and the set of all elements of S will be *C*-sets. So also will be the union of two *C*-sets and the intersection of an arbitrary set of *C*-sets. The *C*-sets will therefore have the properties usually required of closed sets. Every *CB*-set will be a *C*-set, and the lattice of the *CB*-sets will be an ideal within the lattice of the *C*-sets.

A topological space S will be said to be *bounded* if the set of all its points is bounded. If S is bounded its *C*-sets will be indistinguishable from its *CB*-sets. A bounded, topological space in the revised sense of the term will thus be equivalent to a Kuratowski space in the classical sense.[1] Every space S will determine a bounded space S' such that the points of S' are the points of S and the *CB*-sets of S' the *C*-sets of S. However, the properties of S will not be deducible from those of S', since it is easy to show that S is not uniquely determined by S'. From our revised point of view, we shall think of the space R^n of n real variables as an unbounded space, such that the *C*-sets and *CB*-sets of R^n are its closed and closed, bounded sets, respectively, defined in the usual manner. (This is a different topological entity from the space R^n regarded as a Kuratowski space where all closed sets are treated on an equal footing.)

The *closure* of a set A will be, as usual, the intersection of all the C-sets containing A. The set A will be *bounded* if its closure is a CB-set. The *O-sets* (open sets) will be the complements of the closed sets, the *OB-sets* (open, bounded sets) will be the open sets such that their closures are CB-sets. A space S will be called a *T_0-space* provided that if P and Q are any two distinct points of S there exists an OB-set containing one but not both of the points. The space will be called a *T_1-space* provided there exists an OB-set containing P but not Q. Finally, the space will be called a *T_2-space*, or *Hausdorff space*, provided there exists an OB-set containing P and such that the closure of the set does not contain Q. If the space S is bounded, these definitions will evidently be equivalent to the classical ones.[2]

A set $[A]$ of sets will be said to have the *finite intersection property* if the intersection of each finite subset of $[A]$ contains at least one point. A space S will be said to be *semi-bicompact* provided that if $[A]$ is any set of CB-sets with the finite intersection property there is at least one point of S common to all the sets A of $[A]$.[3] A space S will be said to be *bicompact* if it is both semi-bicompact and bounded. This definition is again in agreement with the classical one. By a mere paraphrase of Wallman's bicompactification process,[4] every T_1-space S may be imbedded in a semi-bicompact space S^*, which last will reduce to S itself if S is initially semi-bicompact. Thus the process as applied to the space R^n of n real variables (interpreted as a space of the new sort) will simply imbed R^n in itself and not in a bicompact space.

As usual, a transformation will be *continuous* provided the original of every C-set is a C-set. A transformation will be *completely continuous* if it is continuous and if, moreover, the image of every CB-set is a CB-set. Two spaces will be *homeomorphic* if there exists a one-one completely continuous transformation of the first space into the second such that its inverse is also completely continuous. Such a transformation will pair the points of one space with the points of the other and the CB-sets of one space with the CB-sets of the other (hence, automatically, the C-sets).

The theory of connectivity in terms of gratings[5] assumes a very satisfactory form as a result of the above generalizations. We take as the covering pairs of S all ordered pairs (a, c) such that a is a CB-set and c a C-set, thus making a distinction between the sets a and the sets c. The barriers b of the covering sets will automatically be CB-sets. The chains such that their loci are CB-sets will now play the rôle of the so-called bicompact chains appearing in the original presentation of the theory.

[1] Cf., for example, Alexandroff and Hopf, *Topologie*, p. 37.

[2] Loc. cit., pp. 58, et seq.

[3] In my paper on "A Theory of Connectivity in Terms of Gratings," *Annals of Mathematics*, **39**, 883–912 (1938), the term "locally bicompact" should be replaced throughout

by the term "semi-bicompact," as suggested in the footnote on p. 885. A semi-bicompact T_1-space is obviously locally bicompact in the usual sense.

[4] H. Wallman, "Lattices and Topological Spaces," *Annals of Mathematics*, **39**, 112–126 (1938).

[5] See footnote 3. In connection with the paper there referred to, the following addendum may be useful. Throughout the paper, no mention is made of the so-called *dual connectivity theory* which follows along the lines developed by Vietoris and extended to general spaces by Čech. This is because the dual theory follows immediately from the more powerful direct theory by purely group theoretical considerations. The chains of a space S (before reduction modulo the empty chains) are expressible as finite polynomials in the a_i's and b_i's such that no index i appears more than once in any term. They, therefore, form a free group G with a generator corresponding to each possible term. A character of G is thus determined when we arbitrarily assign its value at each generator of G. It can, therefore, be represented by an infinite polynomial in the a_i's and b_i's (with no repeated index in any term), such that the coefficient of each term is a real number reduced modulo 1, equal to the value of the characters at the generator of G corresponding to the term. The group H of all such infinite polynomials is the character group of G. From this point on, the theory of the dual connectivity groups can be developed along the lines suggested by Pontrjagin.

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CONTENTS

Page

BOTANY.—VITAMIN B_1, A GROWTH SUBSTANCE FOR EXCISED TOMATO ROOTS. . *By William J. Robbins and Mary Bartley Schmidt* 1

PHYSIOLOGY.—CHEMICAL RESTORATION IN NITELLA. III. EFFECTS OF INORGANIC SALTS. . . . . *By W. J. V. Osterhout and S. E. Hill* 3

PHYSICS.—PHOTOMICROGRAPHS OF THIN BONE SECTIONS BY THE USE OF FLUORESCENCE X-RADIATION . . . . . *By Elmer Dershem* 6

GENETICS.—THE INDUCTION BY IRRADIATION WITH NEUTRONS OF HEREDITARY CHANGES IN MICE . . . . . *By George D. Snell* 11

GENETICS.—IMAGINAL DIFFERENTIATION INAUGURATED BY OXYGEN IN DROSOPHILA PUPAE . . . . . *By Dietrich Bodenstein* 14

CHEMISTRY.—EXPERIMENTS ON THE RELATION OF NUTRITION TO THE COMPOSITION OF THE BODY AND THE LENGTH OF LIFE . . . . . *By H. C. Sherman, H. L. Campbell and C. S. Lanford* 16

PSYCHOLOGY.—THE RESOLUTION OF TESTS INTO TWO GENERAL FACTORS . . *By Edwin B. Wilson and Jane Worcester* 20

PSYCHOLOGY.—THE STEADY POLARITY POTENTIAL OF THE HUMAN EYE . . . *By Walter R. Miles* 25

ASTRONOMY.—THE PHYSICAL STATE OF THE INTERSTELLAR GAS CLOUDS . . *By Otto Struve* 36

MATHEMATICS.—GROUPS OF DEGREE n IN WHICH THE LARGEST DEGREE OF A SUBSTITUTION IS A MINIMUM . . . . . *By G. A. Miller* 43

MATHEMATICS.—EULER'S CONCORDANT FORMS . . . . . *By E. T. Bell* 46

MATHEMATICS.—ON THE MAPPING OF ABSTRACT SPACES ON POLYTOPES. . . *By S. Lefschetz* 49

MATHEMATICS.—THE INTRINSIC METRIC OF A POLYTOPE . *By John W. Tukey* 51

MATHEMATICS.—ON THE CONCEPT OF A TOPOLOGICAL SPACE . . . . . *By J. W. Alexander* 52

ge
1
3
6
11
14
16
20
25
36
43
46
49
51
52